2003

Characteristics of fat free yogurt as influenced by the incorporation of folic acid

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CHARACTERISTICS OF FAT-FREE YOGURT AS INFLUENCED BY THE INCORPORATION OF FOLIC ACID

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Dairy Science

by
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B.S. Louisiana State University, 1992
M.S. Louisiana State University, 1997
August, 2003

ACKNOWLEDGMENTS

The author wishes to thank the Lord Jesus Christ for His guidance and support during his undergraduate and graduate endeavors. "For God so loved the world that He gave His only begotten Son, that whosoever shall believe on Him shall have eternal life." John 3:16.

The author would like to acknowledge the assistance of his wife, Zsa Zsa Boeneke for all her love, help, understanding, and support; and his sons, Nicholas and Timothy for their support.

The author would also like to acknowledge the assistance of other persons who have helped in this endeavor. He would like to thank Dr. Kayanush Aryana for his continual guidance, patience, and support. He would like to thank workers in the LSU Creamery for their support- namely, Matt Summers and Clint Dyke. Thanks are also extended to his committee members Dr. Bruce Jenny, Dr. Paul Wilson, Dr. Witoon Prinyawiwatkul, and Dr. Maren Hegsted for all of their time and advice.

The author would like to express his gratitude to his parents, Sonny and Dottie Boeneke, his grandparents, Jimmy and Ruth Byers, and his aunt, Carol Byers for their continued love and support.

Thanks are also extended to the Louisiana State University Agricultural and Mechanical College and the Louisiana Agricultural Experiment Station for their financial support of this research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Folic acid fortification is used in the prevention of neural tube defects such as spina bifida and ancencephaly, heart defects, facial clefts, urinary tract abnormalities, and limb deficiencies. Although yogurt is not a good source of folic acid, fortification could aid in prevention of above mentioned defects. Fortification of yogurt with folic acid may or may not change its physico-chemical characteristics. Fat free sugar free yogurt was manufactured using 0, 25%, 50%, 75% and 100% of the recommended daily allowance of 400 micrograms of folic acid. Treatments included addition of folic acid at these levels before and after pasteurization. Lemon and strawberry flavorings were added to improve flavor and improve color of yogurts. The objective was to examine the effects of folic acid on viscosity, pH, TA, syneresis, color, composition, and folic acid concentration in the product at one, three, and five week intervals. Data were analyzed using the General Linear Model procedure with a general linear model with repeated measures in time analysis by the Statistical Analysis System. Significant differences were determined at $P<0.05$ using Tukey's Studentized Range Test. There were no differences in the electrophoretic mobilities of the protein/peptides in the samples. Mean flavor scores were higher for lemon and strawberry yogurts as compared to plain when tested by a trained sensory panel. Folic acid fortification of yogurt impacted some of its physico-chemical attributes.

CHAPTER 1: INTRODUCTION-PLAIN YOGURT

A goal of the dairy industry is to increase sales of dairy products. Development of dairy products with new flavors and products with health benefits can achieve this goal. Dairy products in the market place are targeted to different consumer groups. For example, fat free dairy products are targeted to consumers with cardiovascular problems or those trying to lose weight. Lactose free products are targeted to people with lactose intolerance. Consumers are demanding dairy products that taste good and have increased health benefits. Yogurt is a low calorie dairy product which is consumed as a snack and dessert. There has been a steady increase in per capita sales of yogurt from 1588 million pounds in 1996 to 1999 million pounds in 2001 (Milk Facts, 2002). The per capita consumption of yogurt has increased by 7.7% between 2000 and 2001 (Milk Facts, 2002).

Folic acid, a water soluble vitamin, has alternative names such as folate ,folacin, folacid, folbal, PGA, Pterol-L-glutamic acid, Pterol-L-monoglutamic acid, and vitamin B_9 (American Chemical Society, 2002). It exists naturally as a reduced one carbon substituted form of pteroylpolyglutamate (Bailey, 1988). Folic acid contains 3 different parts necessary for its vitamin activity. They include pteridine, para aminobenzoic acid, and glutamic acid (Groff and Grooper, 1998.)

Sources of folic acid include liver, avocado, black beans, spring greens, asparagus, and soybean nuts. Dairy products are not a good source of folic acid. Cow's milk contains 5-7µg/100g folic acid (Renner, 1983, and Scott, 1989). The major form of folate in milk is 5-methyl-tetrahydrofolate. This one of the most stable forms (Forssen et al, 2000). Folic acid itself is not reduced and is the most stable form.

Before folate in foods can be absorbed, it must be converted to its monogultamate form. This is done by conjugases present in the jejunal mucosa, pancreatic juice, and bile (Bender, 1993). The conversion is zinc dependent so any deficiency in zinc will impair its activity and folate absorption (Pfeffer et al, 1997). Folate is transported into cells by means of a carrier driven process (Wagner, 1982). Once inside, it is converted into a polyglutamate form and functions as a coenzyme. Glutamate residues are added to the monoglutamate form by peptide bonds in reactions requiring ATP (Wagner, 1982). Addition of the residues causes the production of different forms of folate coenzymes and traps the folate within the cell (Wagner, 1982). Red blood cells contain folate in the polyglutamate form (Herbert et al, 1994). Body levels of folate are 5-10 mg. The liver is the primary storage organ (Herbert et al, 1994). Folate binding proteins in renal brush border cells and reabsroption by the kidney allow little folate to be excreted (Brody, 1991). However, when the binding capacity is reached, excess folate is excreted and not stored like fat soluble vitamins (Shils et al, 1999). Folate is used by the body to metabolize serine, glycine, methionine, and histidine. It is also used in purine and pyrimidine synthesis.

Studies have shown that folic acid taken by the mother during initial stages of pregnancy can prevent neural tube defects such as spina bifida, ancencephaly, heart defects, facial clefts, urinary tract abnormalities, and limb deficiencies (Hall and Solehdin 1997). For folate to be effective against these abnormalities, increase in its consumption must occur during the periconceptional period. This is about one month before to one month after conception (Institute of Medicine, 1998). The U.S. Center for Disease Control recommends women of child-bearing age to consume 400µg

of folic acid daily to prevent such neural tube defects (CDC 1993). An additional 200µg is added when pregnant and 100 µg when lactating (Yates et al, 1998). The current RDA for children 1-3 years of age is 150ug/day; 300ug/day for children 4-8 years; 300ug/day for children 9-13 years; and 400ug/day for ages 14 to over 70 years (Food and Nutrition Board, 2002) .

Folic acid has also been shown to reduce the risk of colorectal and breast cancers (Langenohl et at, 2001) and has been shown to act as a cofactor for the conversion of the homocysteine to methionine (Maxwell, 2000). This amino acid is a sulphydryl-containing amino acid derived from the demethylation of dietary methionine. Epidemiological studies over the past 30 years have shown increased concentrations of homocysteine to be associated with vascular disease (Maxwell, 2000). For the levels of homocysteine to be lowered, vitamin supplementation of at least 5 mg folic acid per day is needed (Maxwell, 2000). However, folate ingested at greater than 5mg per day can mask pernicious anemia caused from too little vitamin B_{12} intake (Butterworth et al, 1989). Adequate amounts of vitamin B_{12} are needed for the conversion of homocysteine to methionine. Vitamin B_{12} de-methylates N^5 methyl tetrahydrofolate to tetrahydrofolate, the active form of folic acid (Butterworth et al, 1989).

The whey portion of milk contains a folic acid binding protein (Salter et al., 1981) which protects folic acid from degredation, making it stable (Parodi, 1997) and aids in absorption (Coleman et al, 1981). The folate binding protein (FBP) is a minor whey protein that is present at approximately 10 mg/L in bovine milk (Salter et al., 1972). The FBP amino acid sequence was determined by Svendsen et al, (1984). The FBP contains a single polypeptide chain of 222 amino acid residues with eight

disulphide bridges. The FBP contains a protein portion with a molecular weight of 25,700 and approximately 10% of a carbohydrate part bound to asparagine. The total FBA molecular weight is around 30,000 daltons. The FBP binds folate at a pH range of 5.5-8.0 (Ghitis et al, 1969). The FBP is highly resistant to gastric pH and enzymes in the gastric tract and is not broken down during digestion (Salter and Mowlem, 1983). The FBP's molecular weight is almost the same as beta-lactoglobulin and eludes from gels with this protein (Salter et al., 1981). The FBP's are present in large quantities in commercial whey protein and beta-lactoglobulin preparations (Waxman and Schreiber, 1975). The FBP is temperature stable. The FBP isolated from whey powder obtained from milk vacuum evaporated at 68° C and spray dried at 180° C was able to bind 0.5 mol folate/mol (Salter et al, 1981 in Parodi, 1997).

The FBP also aids in absorption, regulation, and bioavailability of folate. Coleman et al, (1981), demonstrated that the bound folate in the small intestines of rats was increased more than 2 times that of free folate. Tani and Iwai (1984) demonstrated that rats excreted 4 times less folate in urine samples when fed folate bound to bovine FBP. Thus, demonstrating increased retention and more bioavailability of folate. (Parodi, 1997). Since yogurt contains whey, benefits of folic acid addition could be enhanced. Further research needs to be conducted to examine bioavailability of folic acid and FBP's role in its metabolism in dairy products such as yogurt.

Folate bioavailability is not affected by pasteurization (Semchuk et al, 1994). Ristow, et al (1982) found folic acid to be extremely stable at processing temperatures of 120° C for 20 minutes. Andersson and Oste (1992), found folate levels in pasteurized milk not to decrease during storage beyond the expiration date. Wigertz et

at, (1996), reported that yogurts spiked with 5 methyltetrahydrofolate had insignificant differences in the concentration of this vitamin after manufacture. This would make it advantageous to incorporate folic acid in dairy products such as yogurt which must undergo a pasteurization step for their manufacture.

Some yogurt cultures have the ability to synthesize folate. Reddy, et al, (1975) found yogurts inoculated with cultures containing a mixture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were able to synthesize folic acid and increase its content in finished product 10 fold. Various fermented milks have total folate values between 5µg and 18µg/100g (Forssen et al, 2000). A study done by Hoppner et al (1981) reported buttermilk to contain 9.7µg/100g of 5-methyl-tetrahydrofolate(THF). Yogurt contains 4.7µg/100g of 5-methyl-THF (Hoppner et al, 1981). Rao and Shahani (1987) found folate levels in skim milk to decrease from 9.8µg to 1.6µg/100g in 36 hours after inoculation with *Lactobacillus bulgaricus*. Skim milk inoculated with *Streptococcus thermophilus* and *Lactobacillus acidophilus* increased total folate (Rao and Shahani, 1987). In yogurt inoculated with *Lactobacillus bulgaricus* and *Streptococcus thermophilu*s, the *Lactobacillus bulgaricus* probably consumed folates that were made by the *Streptococcus thermophilus* (Rao and Shahani, 1987).

Direct addition of vitamins A and D during fluid milk processing is a common practice. Whether or not the direct addition of a water soluble vitamin, folic acid, during yogurt manufacture would alter the physico-chemical and sensory characteristics of this product are not known. The objectives of this study were:

1. To determine the effect of different concentrations of folic acid on the physico-chemical and sensory characteristics of yogurt over a storage period.

2. To elucidate the effect of the stage of addition of folic acid on the physico-chemical and sensory characteristics of yogurt over a storage period.

CHAPTER 2: MATERIALS AND METHODS

Experimental Design

Plain yogurts were manufactured with 0, 25, 50, 75, and 100% of the RDA of 400µg folic acid per 226 ml cup. Folic acid was added before or after pasteurization of the yogurt mix. Moisture, ash, fat, and protein concentrations were measured at week 1. Folic acid concentration was measured at weeks 1 and 5. Viscosity, pH, titratable acidity, syneresis, color, and sensory analysis were measured at weeks 1, 3 and 5. The experiment was conducted and analyzed as a randomized complete block with repeated measures. The replications were the blocks. Three replications were conducted.

Yogurt Manufacture

Fat free plain set yogurt with no added sugar was manufactured at the Louisiana State University Department of Dairy Science according to Haque and Aryana (2002). Pasteurized skim milk was obtained from Kleinpeter Farms Dairy, Baton Rouge, LA. Non-fat dry milk (NFDM) was obtained from Sunny Meadows, Oklahoma City, OK. Starch (National 46) was obtained from National Starch and Chemical Corp., Bridgewater, NJ. Aspartame was obtained from Jungbunzlauer, Newton Centre, MA. Folic acid powder was obtained from Lakeshore Tech., Norton Shores, MI. Culture (CH3) was obtained from Chrs Hansens, Milwaukee, WI. Yogurt mix formulations are reported in Table1.

Table 1. Fat free plain set yogurt formulas

	Folic acid percentages (RDA)				
Composition	0%	25%	50%	75%	100%
Skim milk	3.78L	3.78L	3.78L	3.78L	3.78L
NFDM	114g	114g	114g	114g	114g
Starch	23g	23g	23g	23g	23g

(Table continued)

Aspartame	1.14g	1.14g	1.14g	1.14g	1.14g
Folic acid	0mg	1.67mg	3.35mg	5.03mg	6.70mg
Starter culture	1.3g	1.3g	1.3g	1.3g	1.3g

Analytical Procedures

Protein concentration was determined on dried yogurt samples. Samples were prepared by drying 15 g of yogurt for 48 hr at 100°C in a convection oven (Fisher Scientific, Houston, TX). Dry sample was ground using mortar and pestle, 0.2 g sample was loaded into tin cups, folded and loaded into a Leco FP428 (Leco Corp., St Joseph, MI) nitrogen analyzer. Sample was incinerated and results expressed as percent nitrogen. Results were multiplied by a protein correction factor of 6.38. Fat, moisture, and ash contents were determined according to (Case, et al, 1985). Folic acid concentration was determined by using high performance liquid chromatography (HPLC) with methods modified from Albala-Hurtado, et al, (1997). The HPLC system was comprised of a Waters (Waters Corp., Milford, MA) 501 pump, Waters 717 Plus auto-sampler, and Waters 486 tunable UV detector set at 282 nm. Peak areas were calculated using the Waters Millinium® software. The separation was carried out isocratically using a Waters Spherisorb 5um ODS2 4.6x250 mm column with guard cartridge. Samples were prepared by dissolving 8 g of yogurt in 10 ml of HPLC grade water, 10.5 g of this sample were weighed into 50 ml centrifuge tubes with screw on caps, 1g of crystalline trichloroacetic acid (TCA) was added and the mixture was shaken for 10 minutes on a mechanical shaker. The mixture was centrifuged at 1250 g for 10 minutes. The supernate was decanted to a 10 ml volumetric flask and 3ml of 4% w/v TCA was added to the solid phase. The mixture was shaken for 10 minutes and centrifuged again at 1250g for 10 minutes. The supernate was then added to volume in the 10 ml volumetric

flasks wrapped in aluminum foil to protect from light. Samples were filtered through a 45 micron filter (Sigma Aldrich, St. Louis, MO) and placed in clear glass HPLC vials protected from light with aluminum foil. Eluent was prepared as described in Albala-Hurtado, et al, (1997). A standard curve (Figure 1) was prepared by dissolving known amounts (1.67 mg, 3.35 mg, 5.03 mg, and 6.70 mg) of folic acid in a gallon of HPLC grade double distilled water i.e. the same amount of folic acid is used in a gallon of yogurt mix. A sample volume of 10µl was injected using an autosampler (Waters Corp., Milford, MA). Run time for samples was 20 minutes using a flow rate of 1 ml per minute. These known concentrations of folic acid solutions were filtered through a 45 micron filter (Sigma Aldrich, St. Louis, MO) and injected using an autosampler. Under the conditions used in this experiment, folic acid is known to elute out of the column and be detected by the uv detector at 12-14 minutes (Albala-Hurtado, et al, 1997). Folic acid peak areas corresponding to its known concentrations were used to construct a standard curve. Peak areas of folic acid in yogurt samples were fitted to the standard curve and corresponding values in mg/L were recorded.

Viscosity was measured at 21° C using a Brookfield DVII+ viscometer with helipath stand. The spindle used was a T C spindle set at 30 rpm. Data points (50 per sample) were collected using Wingather® software (Brookfield Engineering Lab, Stoughton, MA).

The pH was measured using an Orion model 250 A / 610 pH meter (Fisher Scientific Instruments, Pittsburgh, PA) which was calibrated prior to use by commercial pH 4.00 and 7.00 buffers(Fisher Scientific). Titratable acidity (TA) was measured by using 9g of yogurt and 0.5ml of phenolphthalein indicator. The mixture was

then titrated using 0.1N NaOH until color changed to pink and persisted for 30 seconds. The TA expressed as percent lactic acid was read off the burette of the NAFIS bottle.

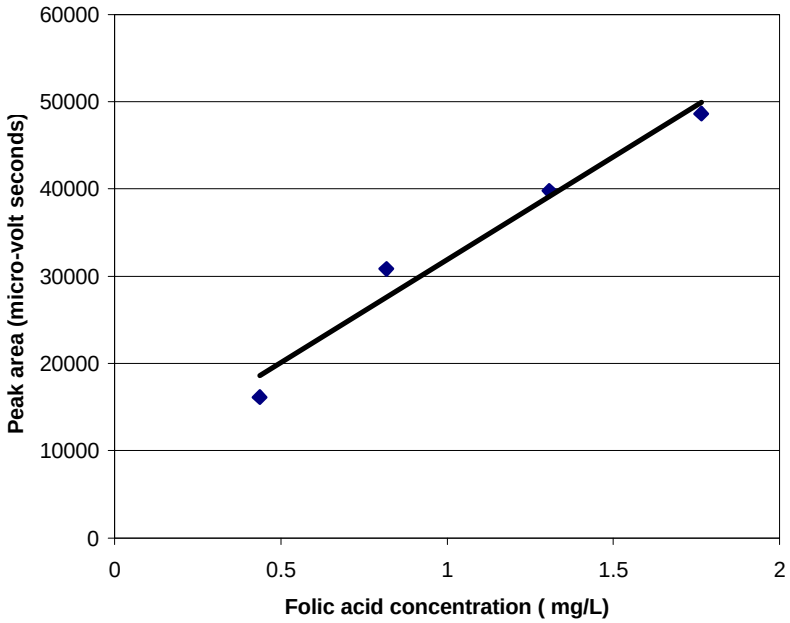


Figure 1. Standard curve of folic acid concentrations

Syneresis was determined by emptying 295g of yogurt into a cheese cloth lined funnel placed on top of a graduated cylinder. Amount of drained of whey in ml was measured at the end of a two hour period at 21° C.

The protein profile was studied by polyacrylamide gel electrophoresis on a Novex XCell Mini Cell (Novex, San Diego, CA) using 4-12% NuPage gels (Novex, San Diego, CA). Samples were prepared by dissolving 0.2g yogurt in 1ml distilled water, 27.9µl of mixture was added to 31.1µl distilled water and 25µl of 4X buffer containing 2% lithium dodecyl sulfate (Novex, San Diego, CA). Samples were heated in a 70° C water bath for 10 minutes. Ten µl of 10X reducing agent containing 500mM dithiothreitol (Novex, San Diego, CA) was added to each sample. Samples were vortexed for 5 sec. Twenty µl of each sample was loaded into each well of the gel.

Running buffers were prepared by adding 50 ml of 3-N-morpholino propane sulfonic acid (MOPS) to 950 ml of distilled water, 600 ml was put in the lower, outer chamber while 200 ml was placed in the upper chamber. Before the upper chamber was filled, 500µl of antioxidant (Novex, San Diego, CA) was added to the 200 ml buffer solution. Two gels were run simultaneously at 400 volts for 1 hour. Gels were taken out of carriage and placed in staining trays containing 110ml distilled water, 40ml methanol, and 40ml stain A containing ammonium sulfate and phosphoric acid (Novex, San Diego, CA) for 10 minutes, followed by a 10ml addition of stain B (Novex, San Diego, CA). Gels stained for 12-14 hrs followed by destaining in distilled water for one week. Gels were scanned using an Hewlett Packard Scan Jet 5300C flat bed scanner (Hewlett Packard, Boise, ID) and images were recorded.

Color was determined by L*a*b* and L*C*h values obtained using a handheld Minolta CM 508 d colorimeter (Minolta Labs, Japan). An average of 5 readings per sample were recorded.

Sensory scoring was conducted in the sensory evaluation room in the LSU Creamery by a three member experienced panel using the official American Dairy Science Association intercollegiate dairy products evaluation score card.

Statistical Anlaysis

Data from, HPLC, viscosity, pH, TA, syneresis, color, and sensory analysis were analyzed by the Statistical Analysis System using the General Linear Model procedure with a repeated measure in time. Data from moisture, ash, and protein concentration were analyzed using the General Linear Model with Tukey's Studentized Range Test. Significant differences were determined at $P<0.05$.

CHAPTER 3: RESULTS AND DISCUSSION

Composition of the yogurts is shown in Table 2. Protein contents on dry matter basis ranged from 36.92 to 42.69%. Fat content for all samples were <0.5%. Moisture content ranged from 84.447 to 90.680%. Ash content ranged from 0.82117 to 0.94937 %. There were no significant differences found for protein, fat, moisture or ash.

Table 2. Mean protein, fat, moisture, and ash for fat free plain set yogurts

Treatments	Protein*	Fat	Moisture	Ash
	%wt./vol.			
1	36.92[a]	<0.5[a]	88.730[a]	0.82[a]
2	40.56[a]	<0.5[a]	88.743[a]	0.83[a]
3	38.09[a]	<0.5[a]	85.827[a]	0.87[a]
4	41.50[a]	<0.5[a]	84.487[a]	0.86[a]
5	42.69[a]	<0.5[a]	90.680[a]	0.87[a]
6	38.12[a]	<0.5[a]	90.397[a]	0.94[a]
7	40.77[a]	<0.5[a]	87.183[a]	0.84[a]
8	38.45[a]	<0.5[a]	90.110[a]	0.85[a]
9	39.07[a]	<0.5[a]	86.237[a]	0.88[a]

[a] Means followed by the same letter are not significantly different at $p<.05$.
1=control; 2=25% RDA before pasteurization, 3=50%RDA before pasteurization, 4=75%RDA before pasteurization, 5=100%RDA before pasteurization, 6=25%RDA after pasteurization, 7=50%RDA after pasteurization, 8=75%RDA after pasteurization, 9=100% RDA after pasteurization.
*Protein reported on dry matter basis.

Folic acid concentrations as peak areas are reported in Figure 2. Folic acid levels added before verses after pasteurization affected mean peak area values. Mean values appeared lower for folic acid added after pasteurization for the majority of samples. Reports in Groff and Grooper (1998) indicated that there are folic acid losses on cooking. Significant differences were found in overall level of folic acid addition. Peak areas did not increase proportionally as folic acid levels increased. This is perhaps due to folic acid not being dispersed evenly in the yogurt samples. Folic acid appeared as reddish yellow flakes dispersed unevenly throughout each sample. Samples with higher concentrations of folic acid had the majority of the red flakes at the bottom of their

containers meaning settling of folic acid had occurred. There were no significant differences among overall mean peak areas for weeks 1 and 5 indicating no significant losses of folic acid concentration over a 5 week storage period.

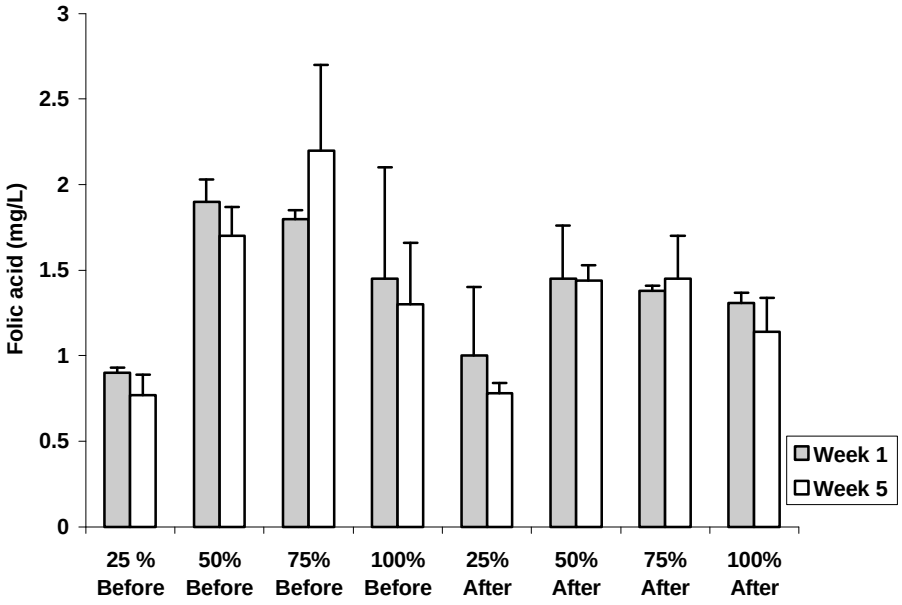


Figure 2. Mean concentration (mg/L) of folic acid in fat free plain set yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

Mean values for viscosity are reported in Figure 3. There were no significant differences in adding folic acid before or after pasteurization of the yogurt mix. Significant differences were found overall for level of folic acid addition. Mean viscosity values appeared to decrease with increasing levels of folic acid indicating an influence of folic acid on viscosity. Increased acid from folic acid addition may have released more whey, causing lower viscosity readings. Overall, no significant differences were found over weeks one, three, and five for mean values of viscosity. Folic acid addition appeared to effect viscosity.

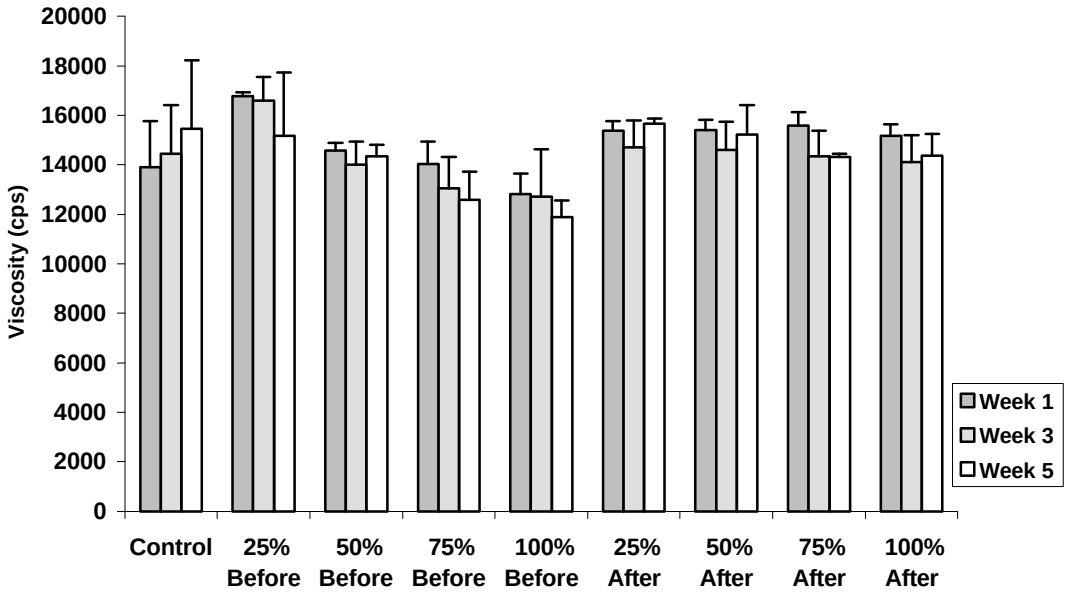


Figure 3. Mean viscosity (cps) of folic acid fortified fat free plain set yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

The pH values are reported in Figure 4. There were no differences in folic acid added before vs. after pasteurization or in level of folic acid concentration. It was expected that folic acid addition might lower pH values; however, no such effect was observed. This is of benefit to the dairy industry since pH is a quality control attribute and incorporation of folic acid did not alter pH. Time overall was found to be significant. This may be due to the culture growing over weeks 1 and 3.

The TA values are reported in Figure 5. There were no significant differences due to stage of addition of folic acid (before vs after pasteurization). Time overall was found to be significant. Changes in TA should have been observed as folic acid concentration increased, but no such effect was observed. This is also of use to the dairy industry since TA is a quality control attribute and incorporation of folic acid did not alter it.

Figure 6 summarizes mean values for syneresis. There were no differences in samples with folic acid added before verses after pasteurization. There were also no significant differences with different concentrations of folic acid. No significant differences in mean values were found overall for weeks one, three, and five. Although differences in viscosity due to increased whey in samples were reported (Figure 3), differences in the amount of whey released as syneresis was not evident (Figure 6).

The electrophoretic migration patterns of proteins/peptides are shown in Figure 7. There were no differences in protein/peptide migration patterns observed. Protein bands in all samples showed separations around 45 kDa and 21.5 kDa. Gels indicated no differences in protein/peptide migration patterns over a five week storage period with addition of all four levels of folic acid before and after pasteurization of the yogurt mixes.

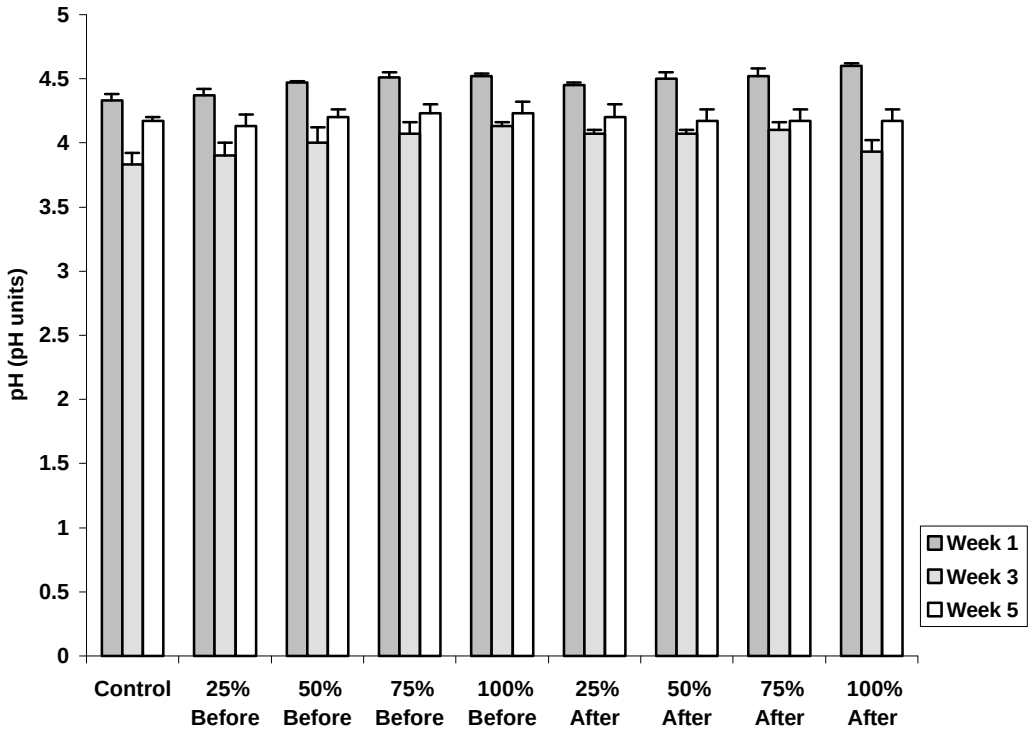


Figure 4. Mean pH of folic acid fortified fat free plain set yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

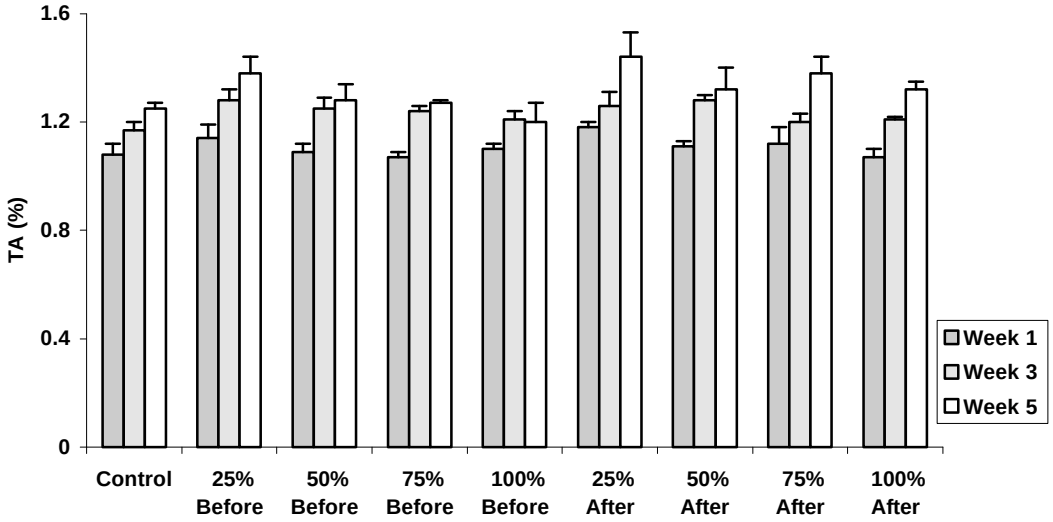


Figure 5. Mean titratable acidity of folic acid fortified fat free plain set yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

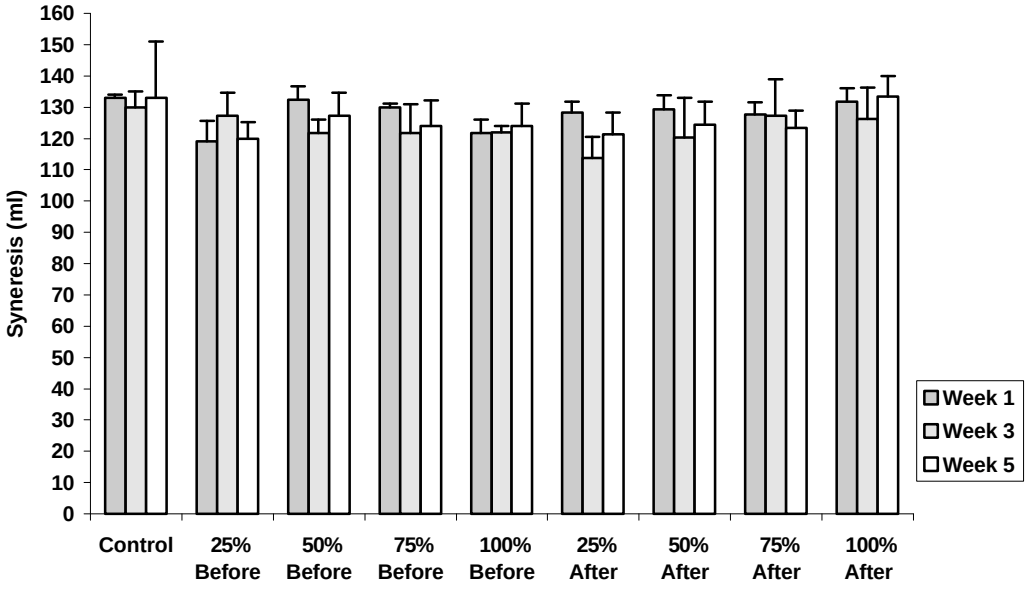
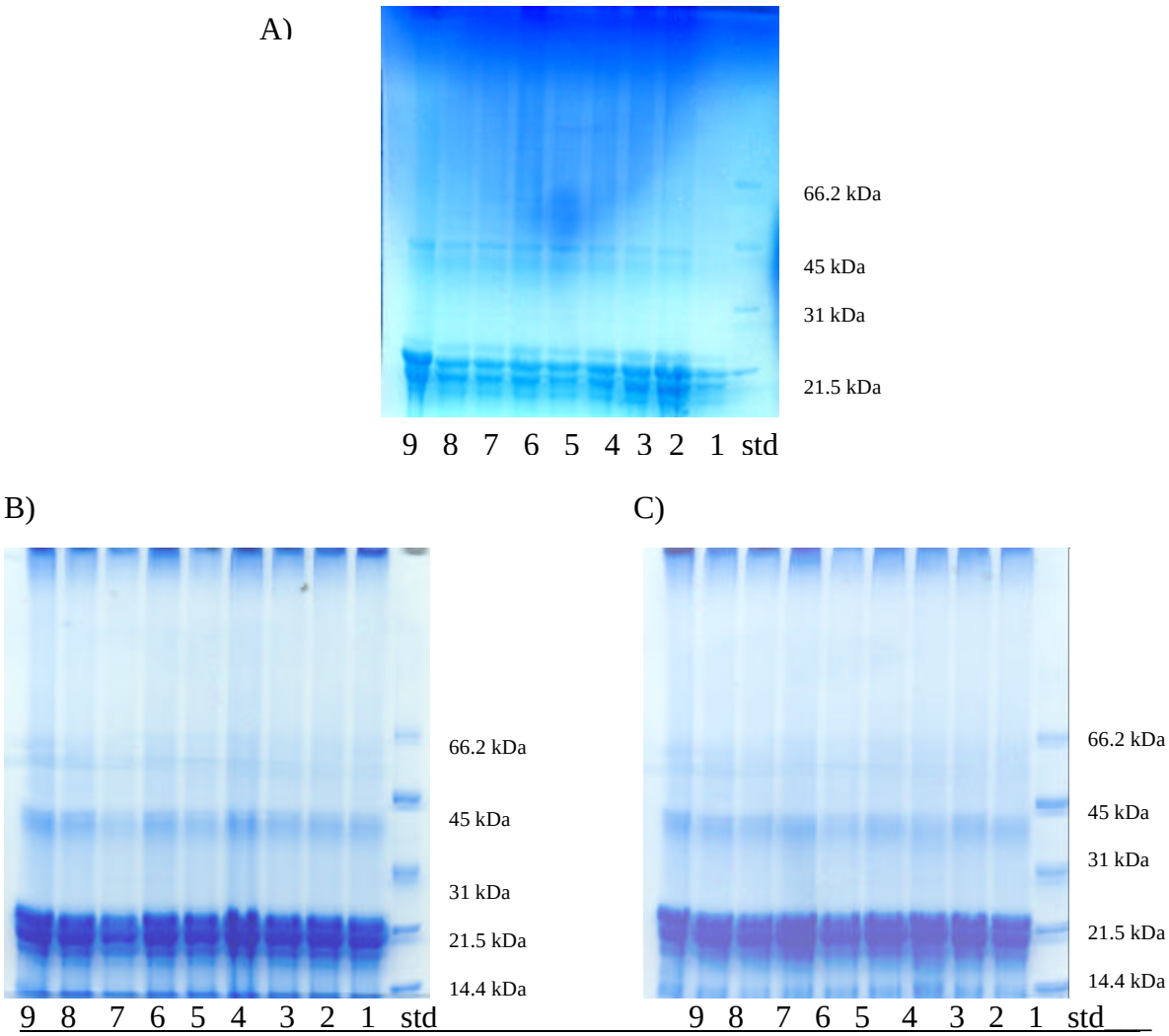


Figure 6. Mean syneresis of folic acid fortified fat free plain set yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization



std=standard; 1=control; 2=25% RDA before pasteurization, 3=50%RDA before pasteurization, 4=75%RDA before pasteurization, 5=100%RDA before pasteurization, 6=25%RDA after pasteurization, 7=50%RDA after pasteurization, 8=75%RDA after pasteurization, 9=100%RDA after pasteurization.

Figure 7. Polyacrylamide gel electrophoresis of fat free plain set yogurts at weeks A) one, B) three, and C) five

The L* (lightness) values are reported in Figure 8. Pasteurization had no significant effect. No overall significant differences in level of folic acid addition were observed in mean L* (lightness) values between samples. Time overall was found to be significant. The L* values were lower at week 3 compared to week 1. The L* values appeared to increase at the end of the 5 week period. This indicates possible

darkening then lightening of color upon storage. The pH values were found to be more acidic at week 3 and less acidic at week 5 compared to week 1 (Figure 4). The increase then decrease in acidity may be related to the darkening and then lightening of samples.

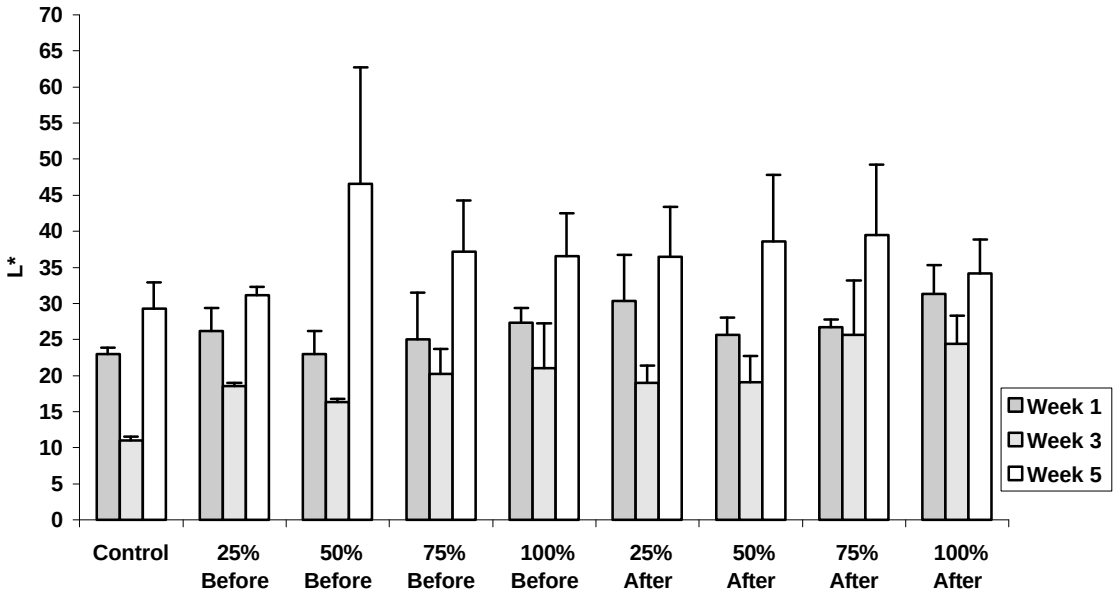


Figure 8. Mean L* values of folic acid fortified fat free plain set yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

Mean a* (redness) values are reported in Figure 9. Pasteurization did not have any effect. There was no significant difference overall in level of folic acid addition among samples. There were, however, differences overall in weeks 1, 3, and 5. Values seemed to decrease at week 3 and increase at week 5 indicating that the samples were less red at week 3 compared to week 5.

The b* (yellowness) values are reported in Figure 10. Pasteurization did not have any effect on b* values. No significant differences were found overall for level of folic acid among samples. However, differences were found overall for time. Mean values at week 5 were lower than values at weeks 1 and 3. This loss of color did not appear to be related to folic acid content. Peak areas from HPLC analysis showed no

significant differences between weeks 1 and 5. Mean values of b* appeared to increase indicating samples appeared more yellow with increasing concentrations of folic acid.

The C* (chroma) values are reported in Figure 11. Overall, there were no significant differences for pasteurization or for level of folic acid addition. No significant differences were found overall in weeks 1, 3, and 5. The level of folic acid addition had no effect on chroma.

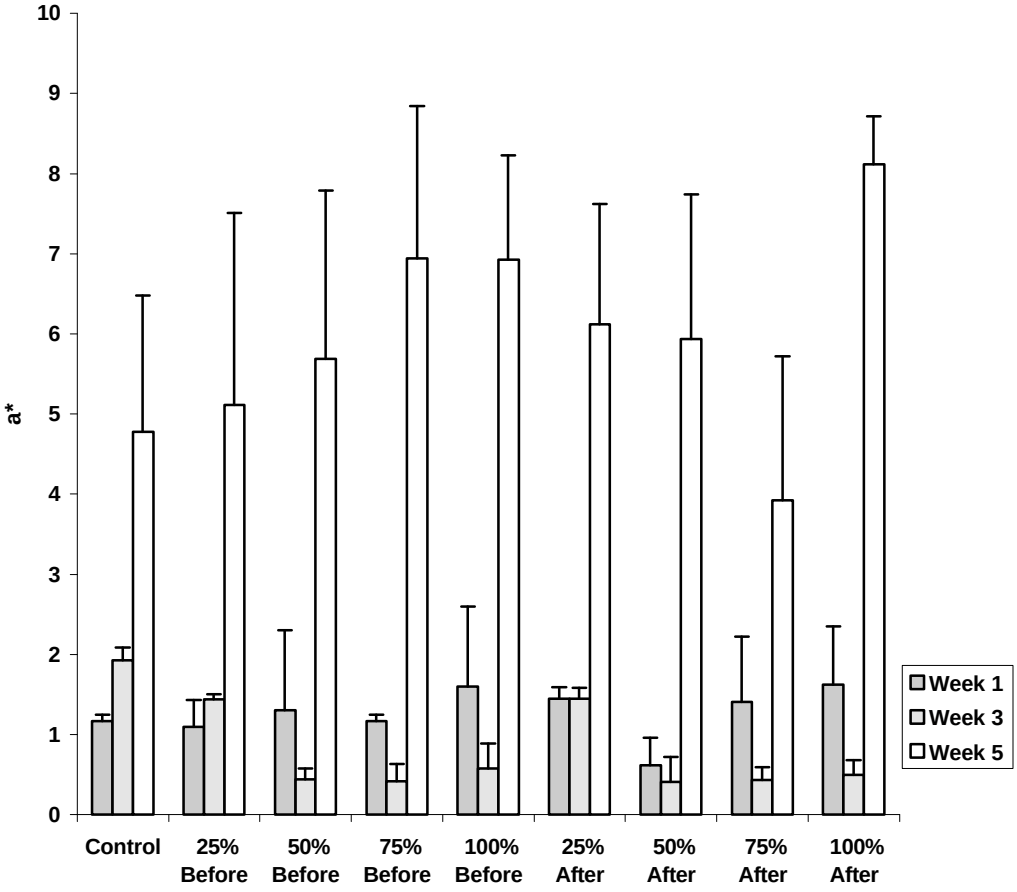


Figure 9. Mean a* values of folic acid fortified fat free plain set yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

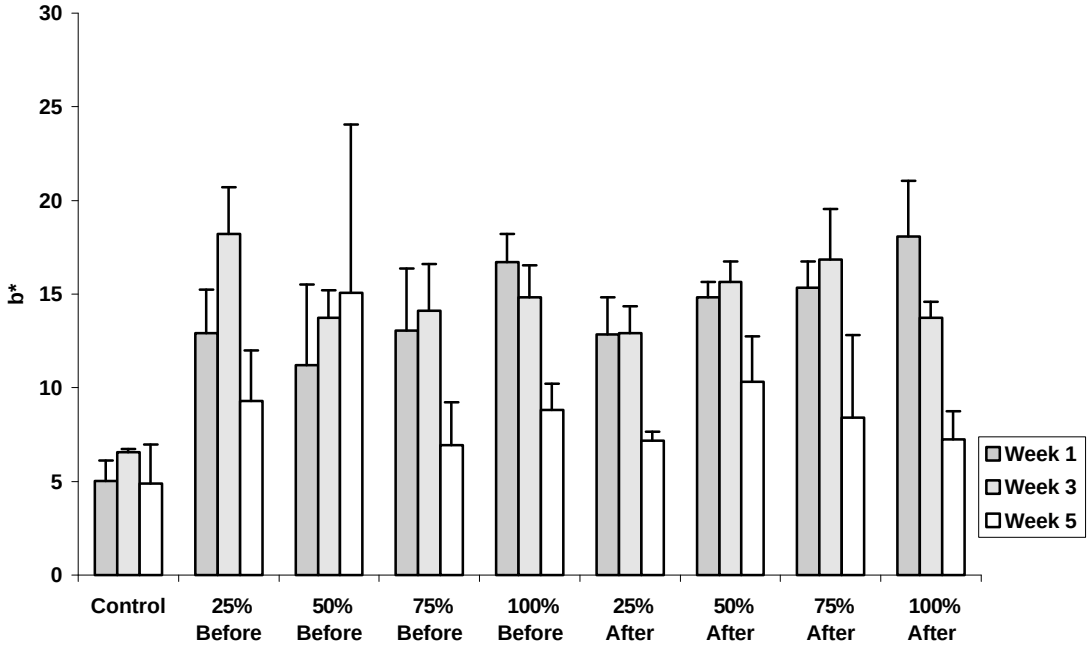


Figure 10. Mean b* values of folic acid fortified fat free plain set yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

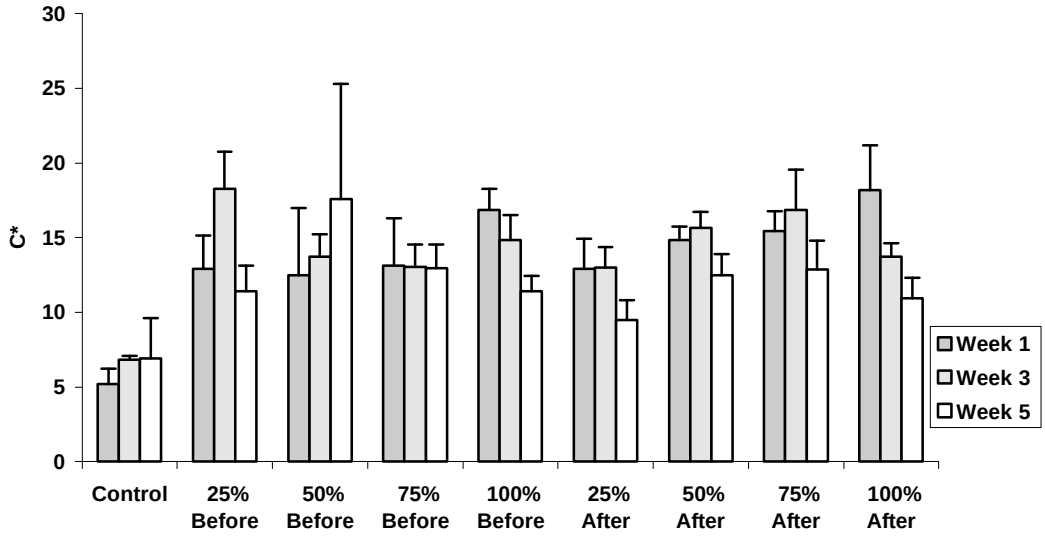


Figure 11. Mean C* values of folic acid fortified fat free plain set yogurt at 25%, 50%, 75%, and 100% RDA before andafterpasteurization

The h (hue angle) values are reported in Figure 12. An h value of 0º is red, 90º is yellow, 180º is green, and 270º is blue. Values for weeks 1 and 3 were close to the 90º indicating a yellow color. At week 5, mean values reached an average of 233 indicating significant changes over the 5 week period. Pasteurization was found to have no effect on this attribute. Folic acid level was found to have overall significant effects. Significant differences were also found overall for weeks 1, 3, and 5.

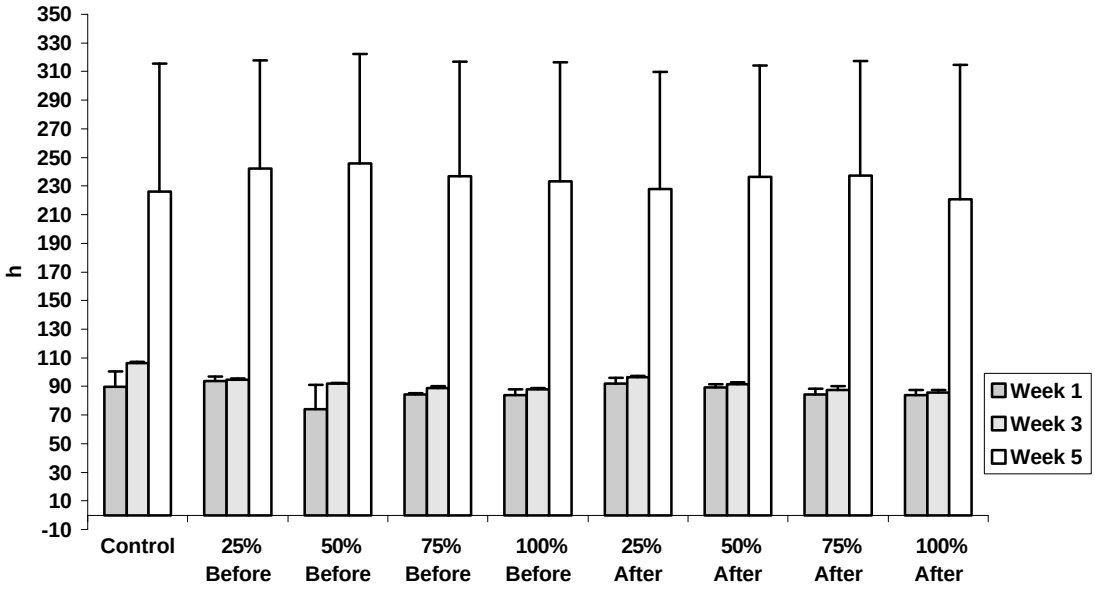


Figure 12. Mean h values of folic acid fortified fat free plain set yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

Mean flavor scores are reported in Figure 13. Flavor scores are from 1 to 10 with 10 being no criticism. No significant differences were found in folic acid addition before vs. after pasteurization. Level of folic acid addition had no overall effect on flavor scores. Mean flavor scores were highest at week 1, then dropped at week 3, and increased again at week 5. This was probably due to acidity of samples. The pH dropped at week 3 then rose slightly at week 5 (Table 5). Flavor of samples are perhaps

preferred most at a pH of around 4.5. Overall flavor of samples fortified with folic acid 50% and over were described as bitter.

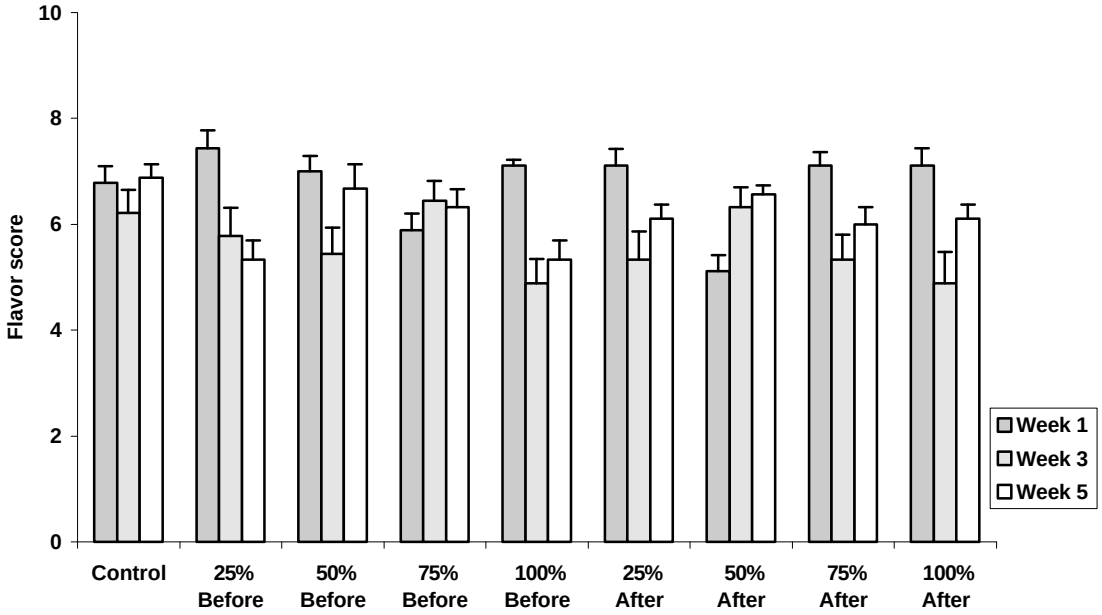


Figure 13. Mean flavor scores of folic acid fortified fat free plain set yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

Mean body and texture scores are reported in Figure 14. Scores are from 1 to 5 with 5 being no criticism. A mean value of 5 indicates no defect in body and texture of yogurts. Most values were 4 and above indicating little defect in body and texture of yogurts. Folic acid addition did not appear to impact body and texture scores. Interaction for time*pasteurization*level was found to be significant.

Mean appearance and color scores are reported in Figure 15. Scores are from 1 to 5 with 5 being no criticism. Time overall for weeks 1, 3, and 5 was significant. An appearance and color score of 5 would show no defect in sample. Mean values showed defects with regard to this attribute. As sample age reached week 5, color changed from the expected yellow color of samples to more of an off yellow color,

impacting sensory appearance and color scores. The interaction pasteurization*level was found to be significant.

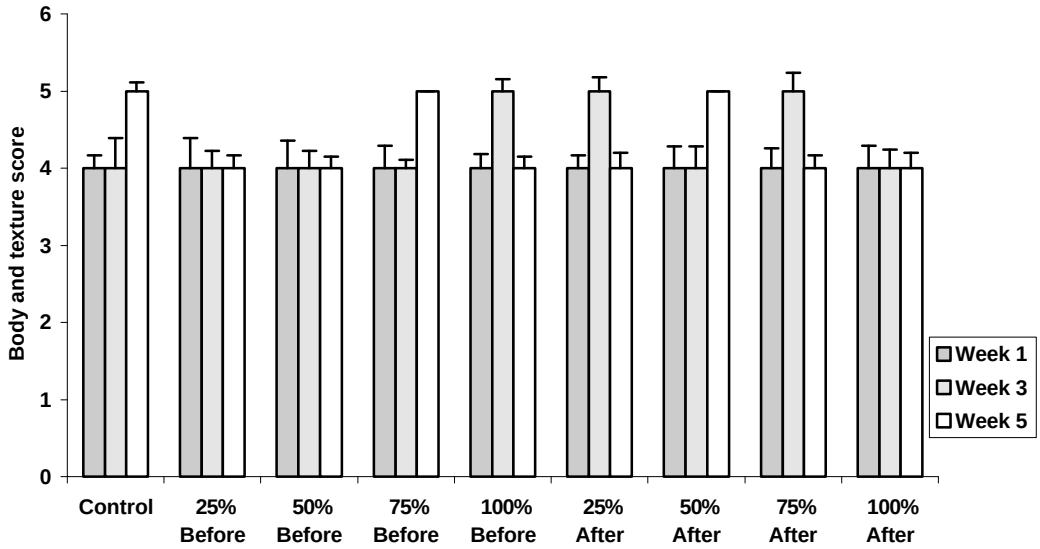


Figure 14. Mean body and texture scores of folic acid fortified fat free plain set yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

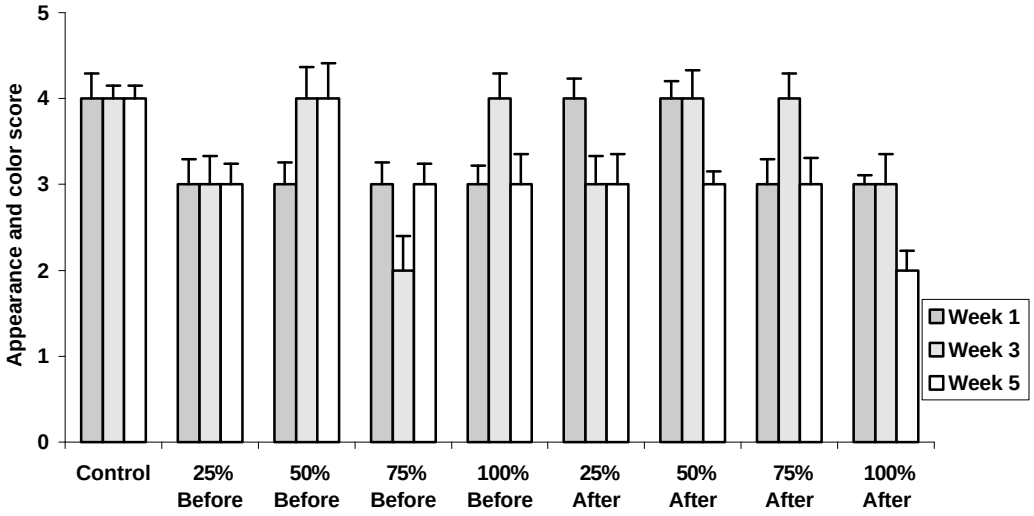


Figure 15. Mean appearance and color scores of folic acid fortified fat free plain set yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

CHAPTER 4: CONCULSIONS- PLAIN YOGURT

Folic acid addition had no effect on protein, fat, moisture, or ash content of yogurts. Samples with folic acid added before pasteurization had higher overall mean folic acid levels than yogurts with folic acid added after pasteurization. Overall folic acid level did not impact flavor or body and texture scores. Folic acid fortification of yogurts would give industry another product in which to fill consumer demand for products that taste good and have health benefits.

CHAPTER 5: INTRODUCTION- FLAVORED YOGURTS

Development of dairy products with new flavors and health benefits help the dairy industry increase sales of products as well as provide consumers with products they enjoy. Dairy products in the market place are targeted to different consumer groups. For example, fat free dairy products are targeted to consumers with cardiovascular problems. Lactose free products are targeted to people with lactose intolerance. Consumers are demanding dairy products that taste good and have increased health benefits. Yogurt is a low calorie dairy product consumed as a snack and dessert. There has been a steady increase in per capita sales of yogurt from 1588 million pounds in 1996 to 1999 million pounds in 2001 (Milk Facts, 2002). The per capita consumption of yogurt has increased by 7.7% between 2000 and 2001 (Milk Facts, 2002).

Direct addition of vitamins A and D during fluid milk processing is a common practice. Whether or not the direct addition of a water soluble vitamin, folic acid, during yogurt manufacture would alter the physico-chemical and sensory characteristics of this product was examined in the previous study. Problems identified were settling and solubility of folic acid in yogurts. This was overcome by altering the pH of folic acid solutions at desired RDA's to 8.27 with NaOH. The other problem identified was the bitter taste. Attempts were made to overcome bitterness by incorporation of flavorings. The next phase of the research was to explore possibilities of correcting the identified problems.

The objectives of this study were:

1. To determine the effect of different concentrations of folic acid on the physico-chemical and sensory characteristics of flavored yogurts over a storage period.

2. To elucidate the effect of the stage of addition of folic acid on the physico-chemical and sensory characteristics of flavored yogurt over a storage period.

CHAPTER 6: MATERIALS AND METHODS

Experimental Design

Lemon and strawberry yogurts were manufactured with 0, 25, 50, 75, and 100% of the RDA of 400µg folic acid per 226 ml cup. Folic acid was added before and after pasteurization of yogurt mix. Moisture, ash, fat, and protein concentrations were measured at week 1 only. Folic acid concentration was measured at weeks 1 and 5. Viscosity, pH, TA, syneresis, color, and sensory analysis were measured at weeks 1, 3 and 5. The experiment was conducted and analyzed as a randomized complete block with repeated measures. The replications were the blocks. Three replications were conducted.

Yogurt Manufacture

Fat free lemon and strawberry flavored yogurts were manufactured at the LSU Dairy Plant according to Haque and Aryana (2002). Pasteurized skim milk was obtained from Kleinpeter Farms Dairy, Baton Rouge, LA. Non-fat dry milk was obtained from Sunny Meadows, Oklahoma City, OK. Starch (National 46) was obtained from National Starch and Chemical Corp., Bridgewater, NJ. Aspartame was obtained from Jungbunzlauer, Newton Centre, MA. Folic acid was obtained from Lakeshore Tech., Norton Shores, MI. Culture (CH3) was obtained from Chrs Hansens, Milwaukee, WI. Lemon and strawberry flavorings were obtained from Target Flavors, Brookfield, CT, and added at a manufacture's suggested usage rate of 9.0 ml/gal and 14.0 ml/gal respectively. Strawberry puree was obtained from Sensient Flavors, Fenton, MO, and was added at a manufacture's suggested usage rate of 20% wt/vol. Solutions of folic acid were made by combining each folic acid RDA amount with 100 ml of water and

adjusting pH to 8.27 with food grade NaOH solution. The yogurt mix formulations are shown in Table 3.

Table 3. Fat free lemon and strawberry flavored yogurt formula

Lemon flavored yogurt formula

	Folic acid percentages (RDA)				
Composition	0%	25%	50%	75%	100%
Skim milk	3.78L	3.78L	3.78L	3.78L	3.78L
NFDM	114g	114g	114g	114g	114g
Starch	23g	23g	23g	23g	23g
Aspartame	1.14g	1.14g	1.14g	1.14g	1.14g
Folic acid	0mg	1.67mg	3.35mg	5.03mg	6.70mg
Water	100ml	100ml	100ml	100ml	100ml
Starter culture	1.3g	1.3g	1.3g	1.3g	1.3g
Lemon flavor	9ml	9ml	9ml	9ml	9ml

Strawberry flavored yogurt formula

	Folic acid percentages (RDA)				
Composition	0%	25%	50%	75%	100%
Skim milk	3.78L	3.78L	3.78L	3.78L	3.78L
NFDM	114g	114g	114g	114g	114g
Starch	23g	23g	23g	23g	23g
Aspartame	1.14g	1.14g	1.14g	1.14g	1.14g
Folic acid	0mg	1.67mg	3.35mg	5.03mg	6.70mg
Water	100ml	100ml	100ml	100ml	100ml
Starter culture	1.3g	1.3g	1.3g	1.3g	1.3g
Straw flavor	14ml	14.ml	14ml	14ml	14ml
Straw puree	73ml	73ml	73ml	73ml	73ml

Straw=strawberry

Analytical Procedures

Protein concentration was determined one week after yogurts were manufactured. Samples were prepared by drying 15 g of sample for 48 hrs at 100°C in a convection oven (Fisher Scientific, Houston, TX). Dry sample was ground using a mortar and pestle, 0.2 g was loaded into tin cups, folded and loaded into a Leco FP428 (Leco Corp., St Joseph, MI) nitrogen analyzer. Sample was incinerated and results expressed as percent nitrogen. Results were multiplied by a protein correction factor of

6.38. Fat, moisture, and ash contents were also determined one week after production according to Standard Methods, 1985. Folic acid concentration was determined by using high performance liquid chromatography with methods modified from S. Albala-Hurtado, et al (1997). The HPLC system was comprised of a Waters (Waters Corp., Milford, MA) 501 pump, Waters 717 Plus auto-sampler, and Waters 486 tunable UV detector set at 282 nm. Peak areas were calculated using the Waters Millinium® software. The separation was carried out isocratically using a Waters Spherisorb 5um ODS2 4.6x250 mm column with guard cartridge. Samples were prepared by dissolving 8 g of yogurt in 10 ml of HPLC grade water, 10.5 g of this sample were weighed into 50 ml centrifuge tubes with screw on caps, 1g of crystalline trichloroacetic acid (TCA) was added and the mixture was shaken for 10 minutes on a mechanical shaker. The mixture was centrifuged at 1250 g for 10 minutes. The supernate was decanted to a 10 ml volumetric flask and 3ml of 4% w/v TCA was added to the solid phase. The mixture was shaken for 10 minutes and centrifuged again at 1250g for 10 minutes. The supernate was then added to volume in the 10 ml volumetric flasks wrapped in aluminum foil to protect from light. Samples were filtered through a 45 micron filter (Sigma Aldrich, St. Louis, MO) and placed in clear glass HPLC vials protected from light with aluminum foil. Eluent was prepared as described in Albala-Hurtado, et al, (1997). A standard curve (Figure 16) was prepared by dissolving known amounts (1.67 mg, 3.35 mg, 5.03 mg, and 6.70 mg) of folic acid in a gallon of HPLC grade double distilled water i.e. the same amount of folic acid is used in a gallon of yogurt mix. A sample volume of 10µl was injected using an autosampler (Waters Corp., Milford, MA). Run time for samples was 20 minutes using a flow rate of 1 ml per minute. These known

concentrations of folic acid solutions were filtered through a 45 micron filter (Sigma Aldrich, St. Louis, MO) and injected using an autosampler. Under the conditions used in this experiment, folic acid is known to elute out of the column and be detected by the uv detector at 12-14 minutes (Albala-Hurtado, et al, 1997). Folic acid peak areas corresponding to its known concentrations were used to construct a standard curve. Peak areas of folic acid in yogurt samples were fitted to the standard curve and corresponding values in mg/L were recorded.

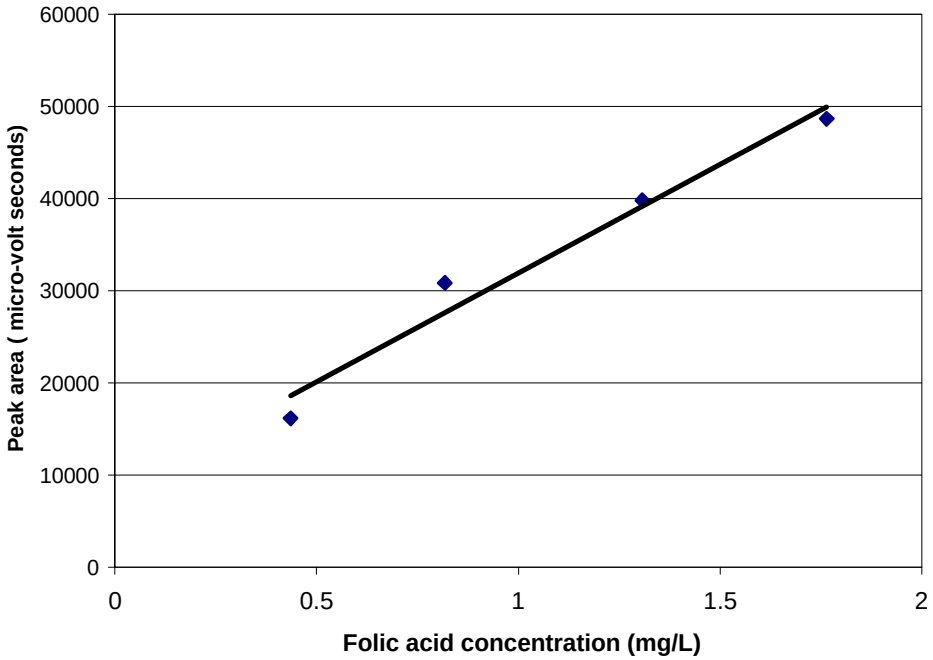


Figure 16. Standard curve of folic acid concentraions

The lemon viscosity was measured at 21° C using a Brookfield DVII+ viscometer with helipath stand. The spindle used was a T C spindle set at 30 rpm. Data points (50) were collected using Wingather® software (Brookfield Engineering Lab, Stoughton, MA).

The strawberry viscosity was measured at 21° C using a Brookfield R/S rheometer with V30-15 vane spindle set at 30 rpm. Data points (50) were collected using RHEO® software (Brookfield Engineering Lab, Stoughton, MA).

The pH was measured using an Orion model 250 A / 610 pH meter (Fisher Scientific Instruments, Pittsburgh, PA) which was calibrated prior to use by commercial pH 4.00 and 7.00 buffers (Fisher Scientific). Titratable acidity (TA) was measured by using 9g of yogurt and 0.5ml of phenolphthalein indicator. The mixture was then titrated using 0.1N NaOH until color changed to pink and persisted for 30 seconds. The TA expressed as percent lactic acid was read off the burette of the NAFIS bottle.

Syneresis was determined by emptying 295g of yogurt into a cheese cloth lined funnel placed on top of a graduated cylinder. Amount of drained of whey in ml was measured at the end of a two hour period at 21° C.

The protein profile was studied by polyacrylamide gel electrophoresis on a Novex XCell Mini Cell (Novex, San Diego, CA) using 4-12% NuPage gels (Novex, San Diego, CA. Samples were prepared by dissolving 0.2g yogurt in 1ml distilled water, 27.9µl of the mixture was added to 31.1µl distilled water and 25µl of 4X buffer containing 2% lithium dodecyl sulfate (Novex, San Diego, CA). Samples were heated in a 70° C water bath for 10 minutes. 10µl of 10X reducing agent containing 500mM dithiothreitol (Novex, San Diego, CA) was added to each sample. Samples were vortexed for 5 sec., and 20µl of each sample was loaded into each well of the gel. Running buffers were prepared by adding 50 ml of 3-N-morpholino propane sulfonic acid (MOPS) to 950 ml of distilled water, 600 ml was put in the lower, outer chamber while 200 ml was placed in the upper chamber. Before the upper chamber was filled, 500µl of

antioxidant (Novex, San Diego, CA) was added to the 200 ml buffer solution. Two gels were run simultaneously at 400 volts for 1 hour. Gels were taken out of carriage and placed in staining trays containing 110ml distilled water, 40ml methanol, and 40ml stain A containing ammonium sulfate and phosphoric acid (Novex, San Diego, CA) for 10 minutes, followed by a 10ml addition of stain B (Novex, San Diego, CA). Gels stained for 12-14 hrs followed by destaining in distilled water for one week. Gels were scanned using an Hewlett Packard Scan Jet 5300C flat bed scanner (Hewlett Packard, Boise, ID) and images were recorded.

Color was determined by L*a*b* and L*C*h values obtained using a handheld Minolta CM 508 d colorimeter (Minolta Labs, Japan). An average of 5 readings per sample were recorded.

Sensory scoring was conducted in the sensory evaluation room in the Louisiana State University Creamery by a 3 member experienced panel using the official American Dairy Science Association intercollegiate dairy products evaluation score card.

Statistical Analysis

Data from lemon yogurts and strawberry yogurts were analyzed separately and in the following manner: HPLC, viscosity, pH, TA, syneresis, color, and sensory analysis were analyzed by the Statistical Analysis System using the General Linear Model procedure with a repeated measure in time. Data from moisture, ash, and protein concentration were analyzed using the General Linear Model with Tukey's Studentized Range Test. Significant differences were determined at $P<0.05$.

CHAPTER 7: RESULTS AND DISCUSSION

The composition of the lemon and strawberry flavored yogurts are reported in Tables 4 and 5 respectively. Protein contents on dry matter basis ranged from 34.09 to 38.38% for lemon yogurts and from 17.64 to 24.41% for strawberry flavored yogurts. Fat content for all samples were <0.5%. Moisture content ranged from 88.46 to 89.77% and 82.04 to 85.58% for lemon and strawberry respectively. Ash content ranged from 0.5667 to 0.9367% and 0.6647 to 0.7139% for lemon and strawberry respectively. No significant differences were found for protein, fat, moisture or ash.

Table 4. Mean protein, fat, moisture, and ash for fat free, sugar free, lemon flavored yogurts

Treatments	Protein*	Fat	Moisture	Ash
	%wt./vol.			
1	34.31[a]	<0.5[a]	88.46[a]	0.89[a]
2	34.09[a]	<0.5[a]	89.36[a]	0.92[a]
3	38.14[a]	<0.5[a]	89.35[a]	0.65[a]
4	38.38[a]	<0.5[a]	89.02[a]	0.94[a]
5	35.82[a]	<0.5[a]	89.34[a]	0.57[a]
6	35.81[a]	<0.5[a]	89.31[a]	0.57[a]
7	36.91[a]	<0.5[a]	89.77[a]	0.73[a]
8	37.28[a]	<0.5[a]	89.67[a]	0.75[a]
9	35.10[a]	<0.5[a]	89.14[a]	0.83[a]

[a] Means followed by the same letter are not significantly different at $p<0.05$.
1=control; 2=25% RDA before pasteurization, 3=50%RDA before pasteurization, 4=75%RDA before pasteurization, 5=100%RDA before pasteurization, 6=25%RDA after pasteurization, 7=50%RDA after pasteurization, 8=75%RDA after pasteurization, 9=100% RDA after pasteurization.
*Protein reported on dry matter basis.

Table 5. Mean protein, fat, moisture, and ash for fat free strawberry flavored yogurts

Treatments	Protein*	Fat	Moisture	Ash
	%wt./vol.			
1	22.06[a]	<0.5[a]	85.58[a]	0.71[a]
2	24.32[a]	<0.5[a]	82.04[a]	0.70[a]
3	22.11[a]	<0.5[a]	85.14[a]	0.71[a]
4	24.41[a]	<0.5[a]	85.09[a]	0.69[a]
5	19.95[a]	<0.5[a]	83.78[a]	0.66[a]

(Table continued)

6	19.44 [a]	<0.5 [a]	84.16 [a]	0.68 [a]
7	21.18 [a]	<0.5 [a]	84.25 [a]	0.69 [a]
8	20.88 [a]	<0.5 [a]	84.15 [a]	0.68 [a]
9	17.64 [a]	<0.5 [a]	82.91 [a]	0.67 [a]

[a] Means followed by the same letter are not significantly different at $p<0.05$.
1=control; 2=25% RDA before pasteurization, 3=50%RDA before pasteurization, 4=75%RDA before pasteurization, 5=100%RDA before pasteurization, 6=25%RDA after pasteurization, 7=50%RDA after pasteurization, 8=75%RDA after pasteurization, 9=100%RDA after pasteurization.
*Protein reported on dry matter basis.

Folic acid peak area concentrations for lemon and strawberry flavored yogurts are reported in Figures 17 and 18 respectively. Pasteurization did not effect folic acid concentrations overall for either lemon or strawberry as it did in the plain. Results described in Groff and Grooper (1998) indicated that there are folic acid losses on cooking. Significant differences were found in overall level of folic acid addition for lemon and strawberry flavored yogurts. Mean values only showed consistent increases between RDA's of 25% and 50%. This is perhaps due to folic acid not being dispersed evenly in the yogurt samples. Although food grade NaOH was used to aid folic acid getting into solution before addition to samples, some settling still occurred. The NaOH aided in the folic acid solubilization in the samples so more accurate HPLC readings could be obtained. No significant differences were found among mean peak areas over weeks 1 and 5 indicating no significant losses of folic acid concentration over a 5 week storage period for lemon yogurts. Interaction for time*pasteurization*level for strawberry flavored yogurts were significant indicating that all three variables-storage time, presence or absence of pasteurization, and level of folic acid collectively impacted the amount of folic acid in the product.

Mean viscosity values for lemon and strawberry yogurts are reported in Figures 19 and 20 respectively. Overall level of folic acid addition was found non significant for both lemon and strawberry yogurts. Pasteurization was found to be

significant for lemon yogurts but not strawberry. Mean values for lemon yogurts were lower for folic acid added after pasteurization. Significant differences were found overall for weeks 1, 3, and 5 for lemon yogurts indicating changes over the 5 week storage period. No differences in storage times were found for strawberry yogurts. Strawberry viscosities were lower than lemon because all strawberry yogurts were stirred uniformly to disperse strawberries before viscosity measurements were taken.

The mean pH values for lemon and strawberry yogurts are shown in Figures 21 and 22 respectively. Level of pasteurization and folic acid addition showed no significant interaction for lemon yogurts. The interaction between pasteurization*level was found significant for strawberry. There were significant differences overall in mean values at weeks 1 and 3 and 5 for lemon and strawberry flavored yogurts. Mean pH values for lemon and strawberry were lower than for plain yogurts. This is probably due to the flavorings added. Folic acid addition did not appear to lower pH of samples. Folic acid addition was expected to lower pH values, but no such effect was observed. This is of use to the dairy industry since pH is a quality control attribute and incorporation of folic acid did not alter pH.

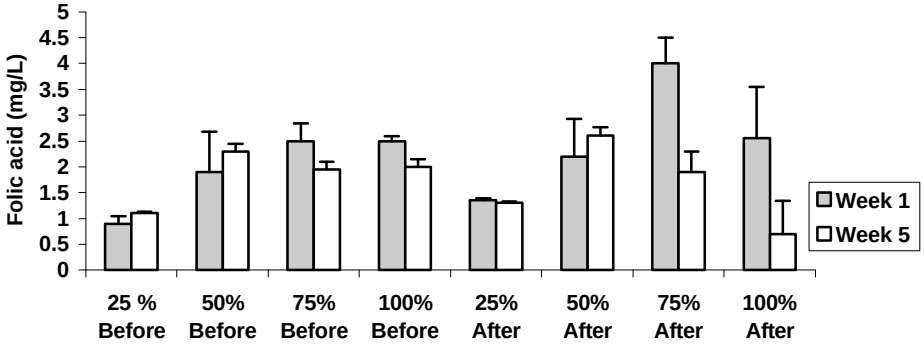


Figure 17. Mean concentration (mg/L) of folic acid in fat free lemon yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

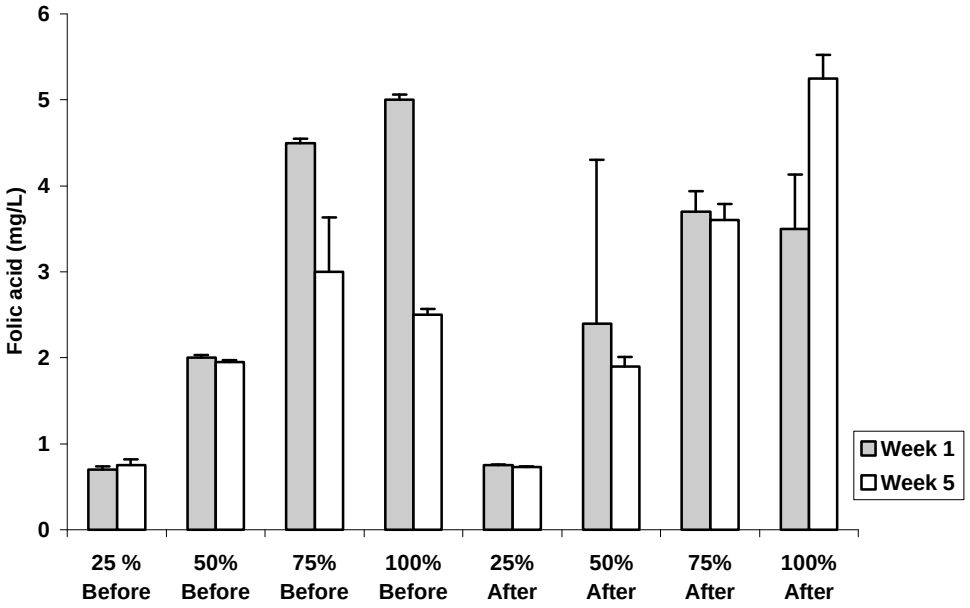


Figure 18. Mean concentration (mg/L) of folic acid in fat free strawberry yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

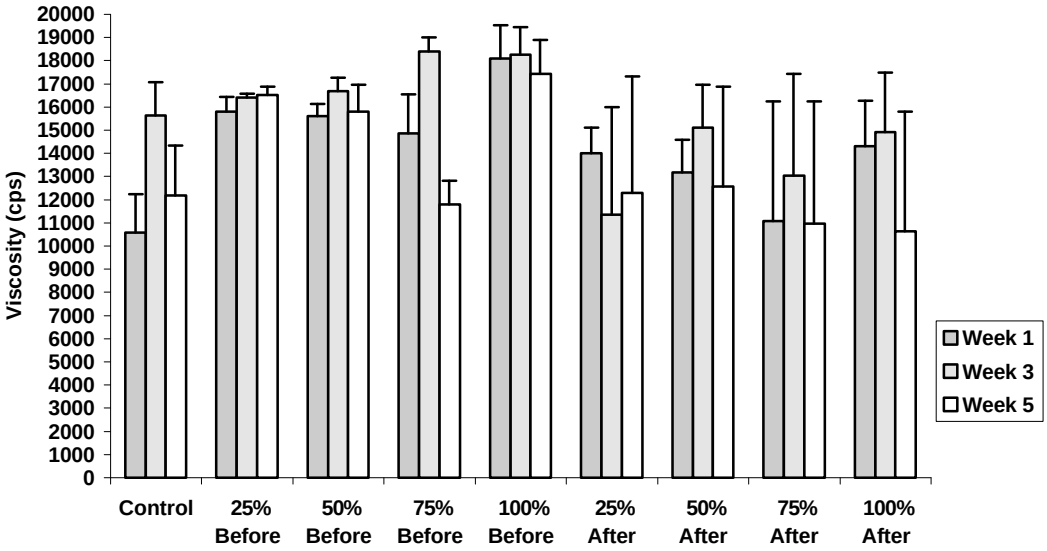


Figure 19. Mean viscosity of folic acid fortified fat free lemon yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

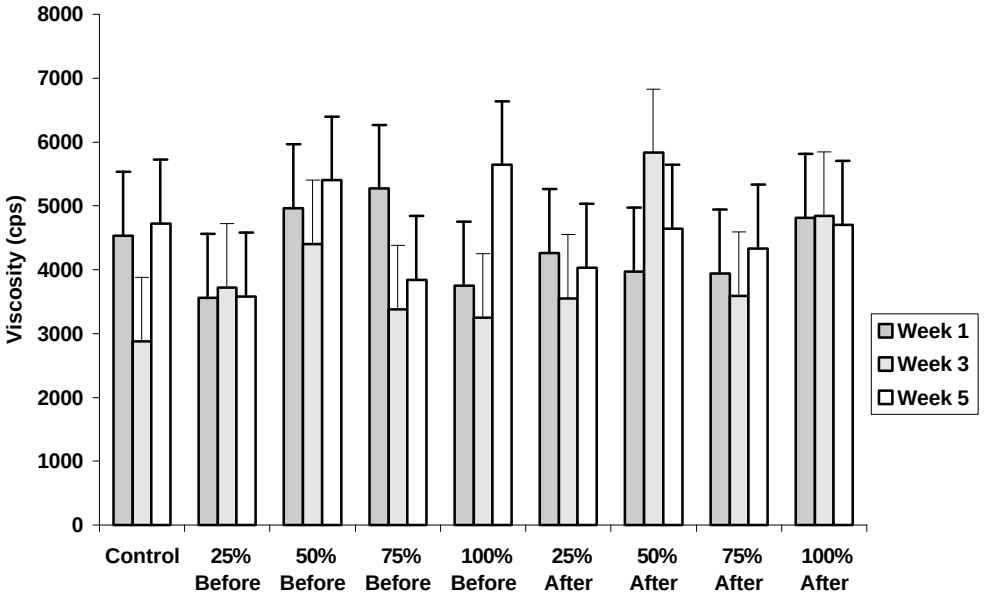


Figure 20. Mean viscosity of folic acid fortified fat free strawberry yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

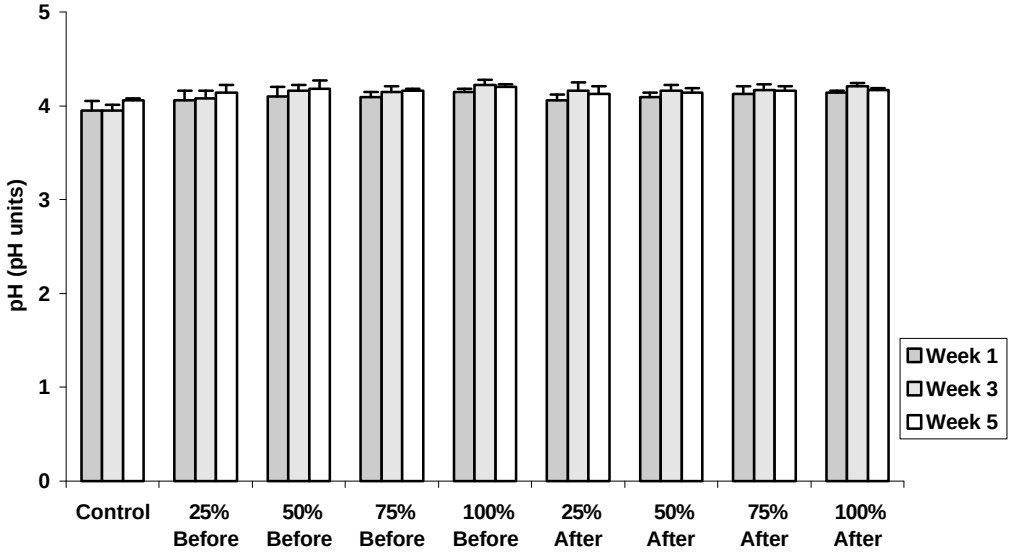


Figure 21. Mean pH of folic acid fortified fat free lemon yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

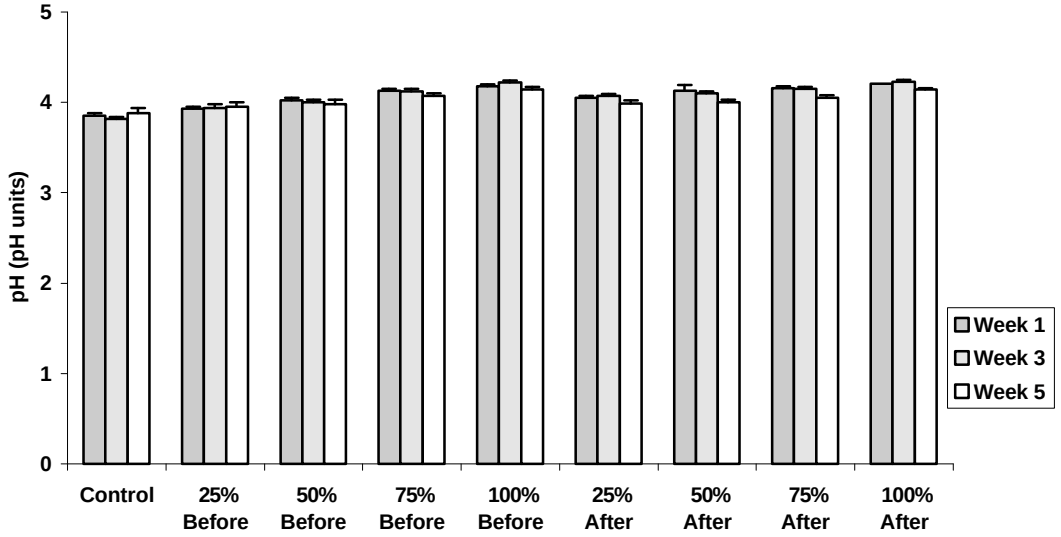


Figure 22. Mean pH of folic acid fortified fat free strawberry yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

The TA values for lemon and strawberry yogurts are reported in Figures 23 and 24 respectively. There were no significant differences due to stage of addition of folic acid (before vs after pasteurization) for lemon or strawberry flavored yogurts. There were no significant differences due to concentrations/levels of folic acid addition overall for weeks 1, 3, and 5 for strawberry, however, differences were found for the lemon. Significant changes in TA should have been observed as folic acid concentration increased. No such effect was observed. This is also of value to the dairy industry since TA is a quality control attribute and folic acid did not affect it.

The mean values for syneresis of lemon and strawberry flavored yogurts are reported in Figures 25 and 26 respectively. Pasteurization, level of folic acid addition and overall time were not significant for strawberry yogurts. Level of folic acid addition was also found not to be significant for lemon yogurts. Significant differences were found in pasteurization and overall time effects for lemon yogurts.

Mean values for syneresis were higher for samples where folic acid was added after pasteurization. Effects were more pronounced in lemon yogurts. Lemon and strawberry flavors were added after pasteurization. Perhaps the acidity of the extracts caused more whey to be separated from samples where folic acid was added after pasteurization. Syneresis findings support viscosity results (Figures 19 and 20). Mean viscosity values were lower for folic acid added after pasteurization in lemon yogurts. Pasteurization and overall time were found to be significant for syneresis of lemon yogurts. No significant interactions were observed for level of folic acid addition in strawberry and lemon yogurts.

Electrophoretic migration patterns of proteins/peptides are shown in Figures 27 and 28 for lemon and strawberry yogurts respectively. There were no differences in protein/peptide migration patterns. Gels indicated no difference in protein/peptide migration patterns over a five week storage period with addition of all levels of folic acid.

The L* (lightness) values for lemon and strawberry yogurts are reported in Figures 29 and 30 respectively. Pasteurization had no significant effect for lemon flavored yogurts, while significant effects were found for pasteurization in strawberry yogurts. No overall significant differences in level of folic acid addition were observed in mean L* values for lemon and strawberry flavored yogurts. Storage time was found to be significant for lemon and strawberry yogurts.

Mean a* (redness) values for lemon and strawberry yogurts are reported in Figures 31 and 32 respectively. Pasteurization was non-significant for both lemon and strawberry yogurts. There was no significant difference overall in level of folic acid addition among strawberry yogurts. Significant differences were found in level of folic

acid added and overall time for lemon yogurts. A significant interaction between time*pasteurization*level was found for strawberry yogurts. Mean a* values for strawberry yogurts were higher than lemon yogurts. This is due to the red color of the strawberries. Red color helped mask yellow color of the folic acid added to the samples.

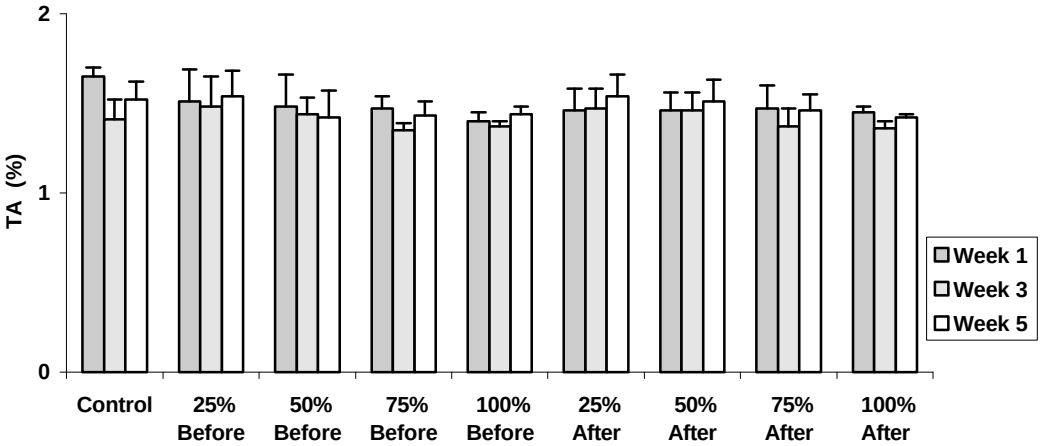


Figure 23. Mean titratable acidity of folic acid fortified fat free lemon yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

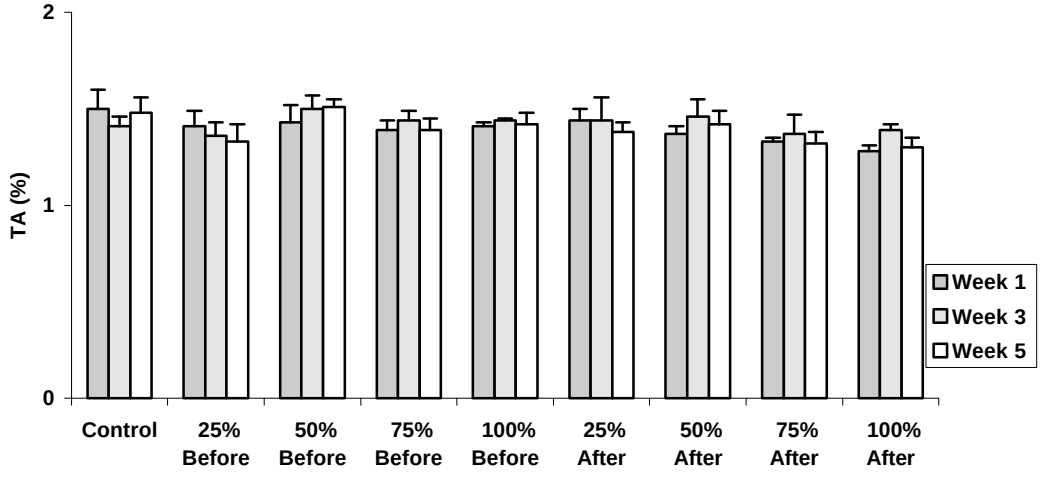


Figure 24. Mean titratable acidity of folic acid fortified fat free strawberry yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

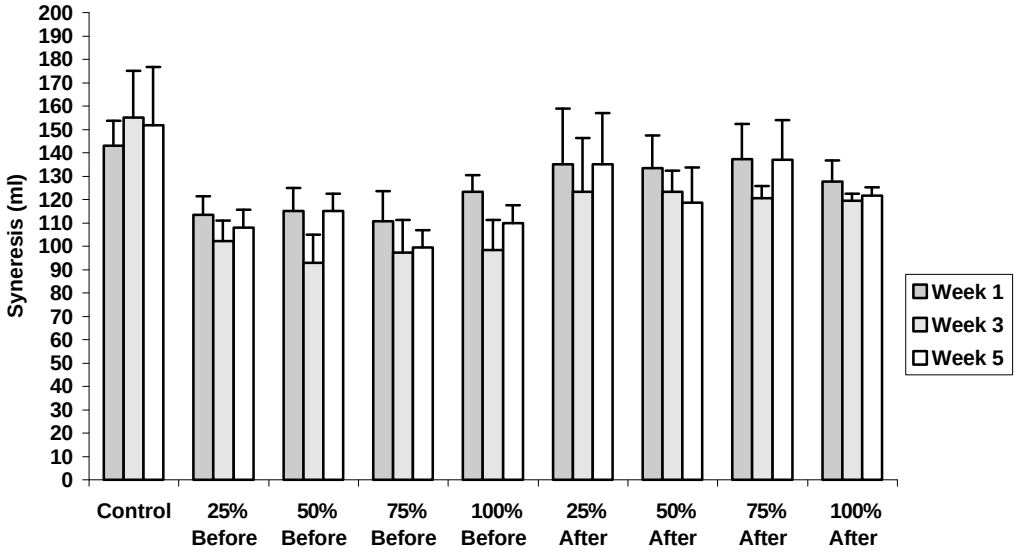


Figure 25. Mean syneresis of folic acid fortified fat free lemon yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

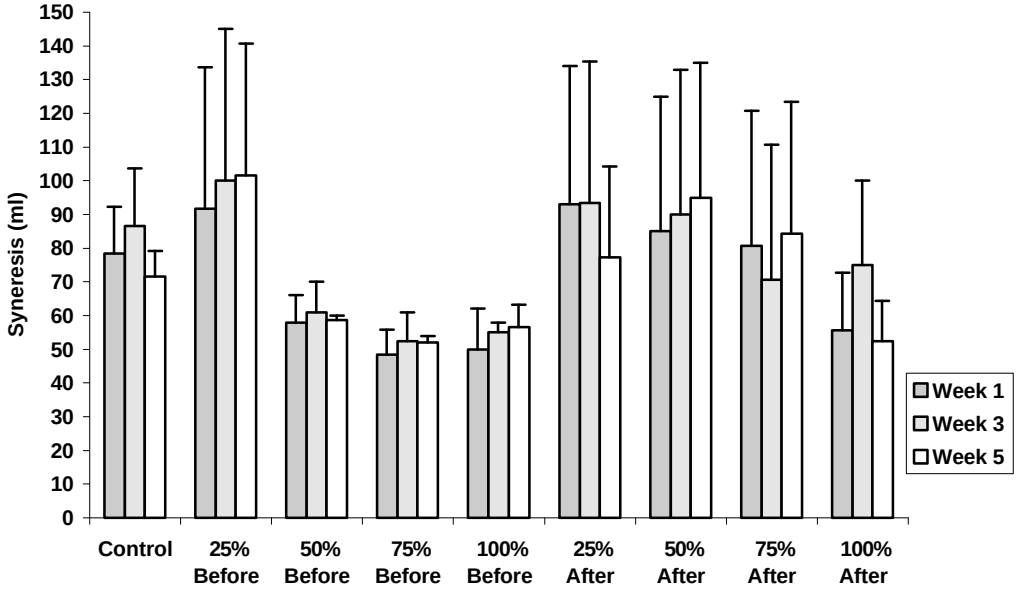
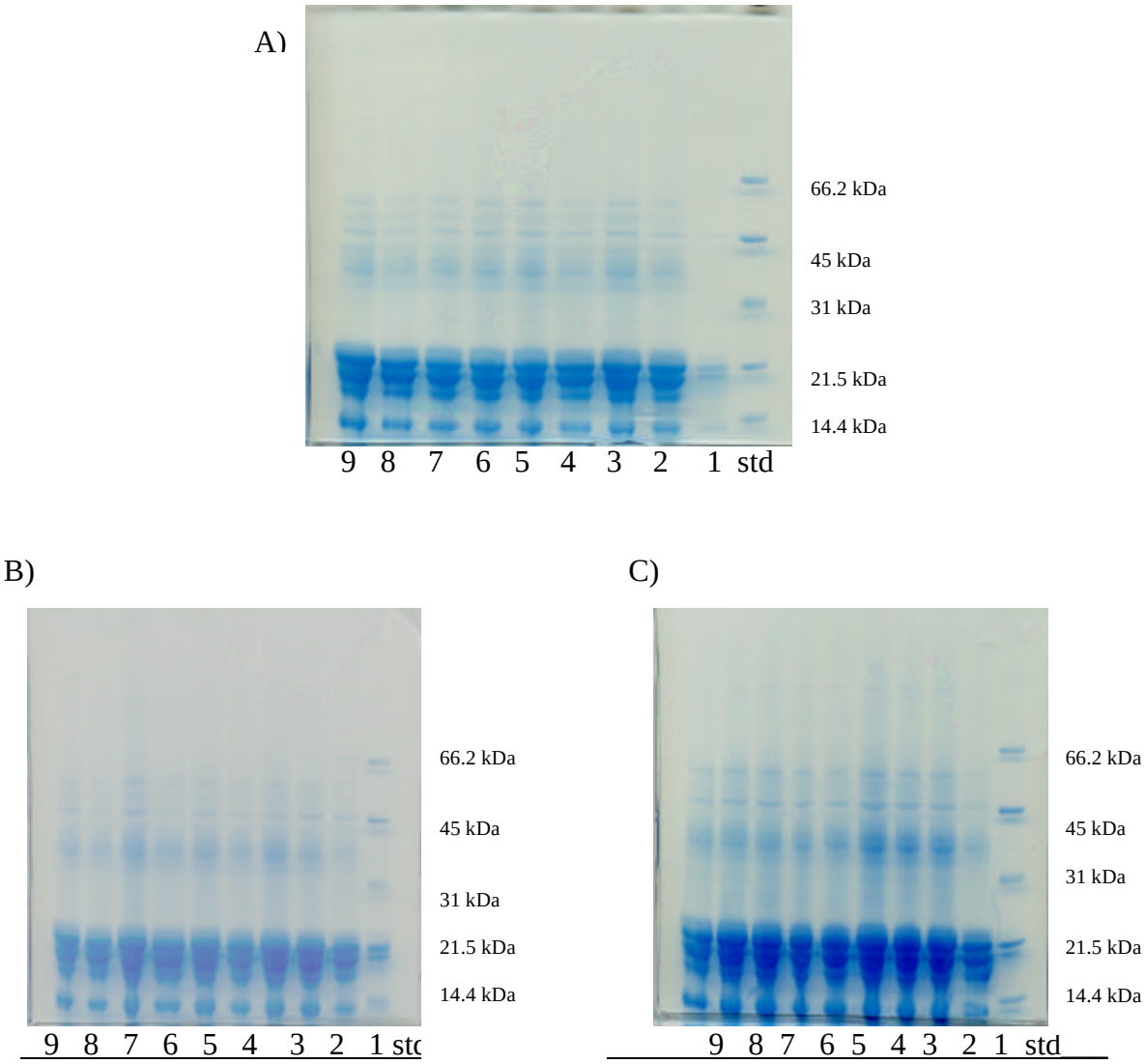


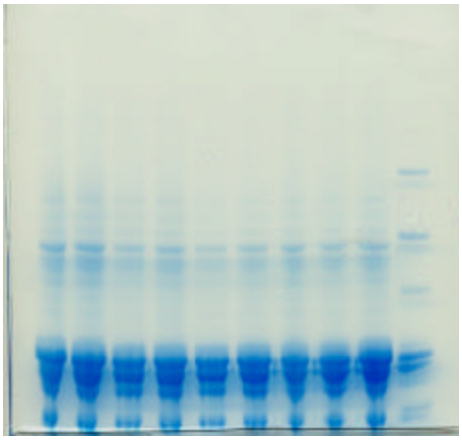
Figure 26. Mean syneresis of folic acid fortified fat free strawberry yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization



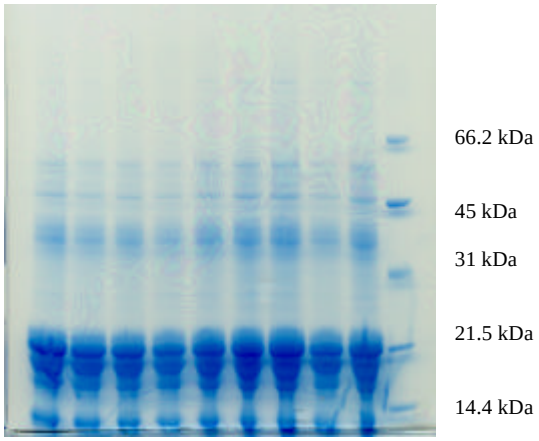
std=standard; 1=control; 2=25% RDA before pasteurization, 3=50%RDA before pasteurization, 4=75%RDA before pasteurization, 5=100%RDA before pasteurization, 6=25%RDA after pasteurization, 7=50%RDA after pasteurization, 8=75%RDA after pasteurization, 9=100%RDA after pasteurization.

Figure 27. Polyacrylamide gel electrophoresis of fat free lemon yogurts at weeks A) one, B) three, and C) five

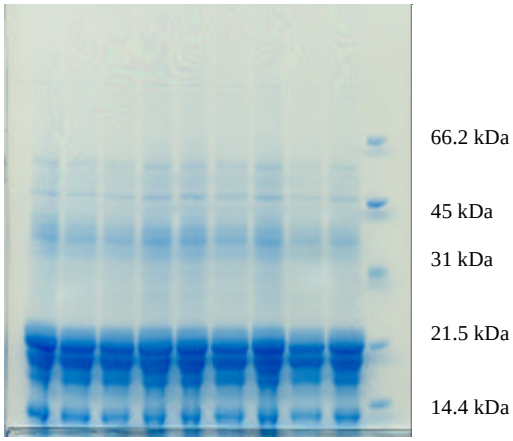
A)



B)



C)



std=standard; 1=control; 2=25% RDA before pasteurization, 3=50%RDA before pasteurization, 4=75%RDA before pasteurization, 5=100%RDA before pasteurization, 6=25%RDA after pasteurization, 7=50%RDA after pasteurization, 8=75%RDA after pasteurization, 9=100%RDA after pasteurization

Figure 28. Polyacrylamide gel electrophoresis of fat free strawberry yogurts at weeks A) one, B) three, and C) five

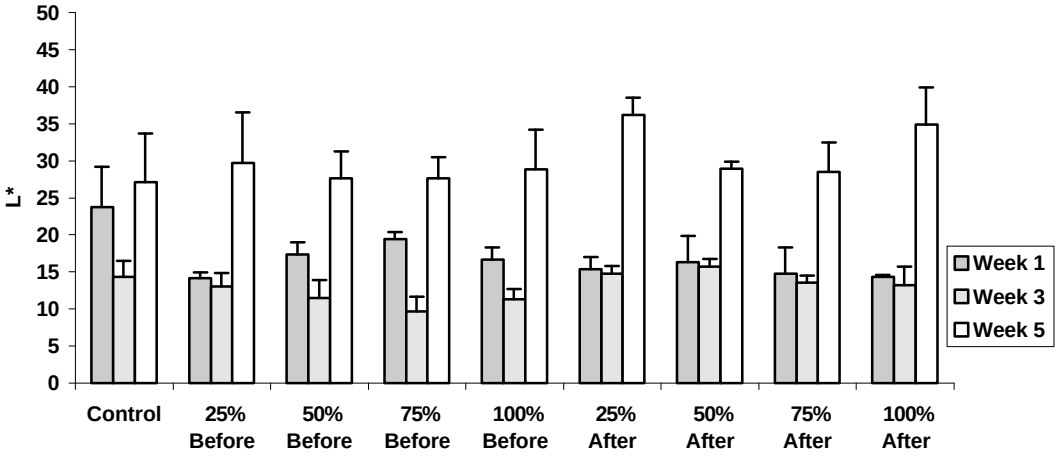


Figure 29. Mean L* values of folic acid fortified fat free lemon yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

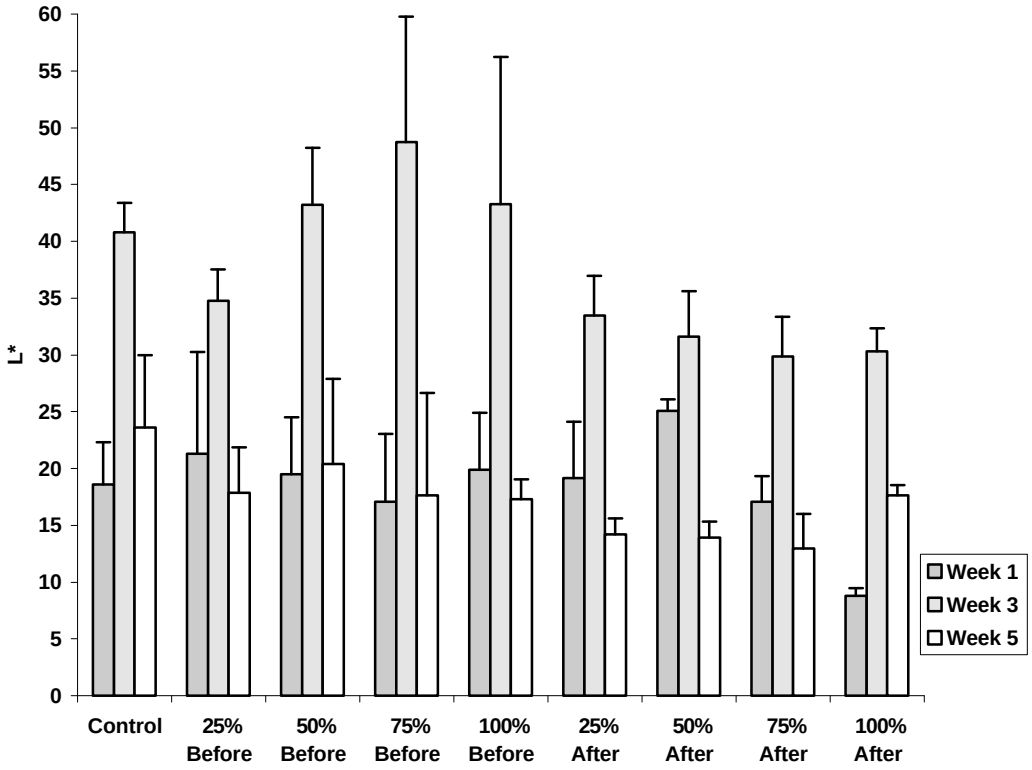


Figure 30. Mean L* values of folic acid fortified fat free strawberry yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

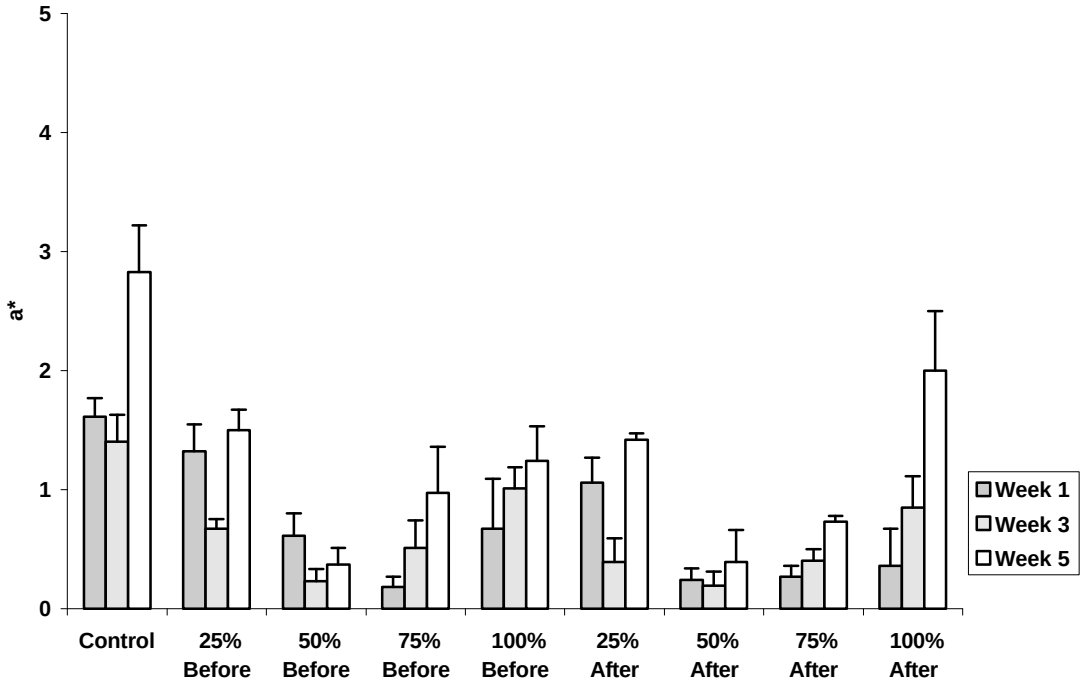


Figure 31. Mean a* values of folic acid fortified fat free lemon yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

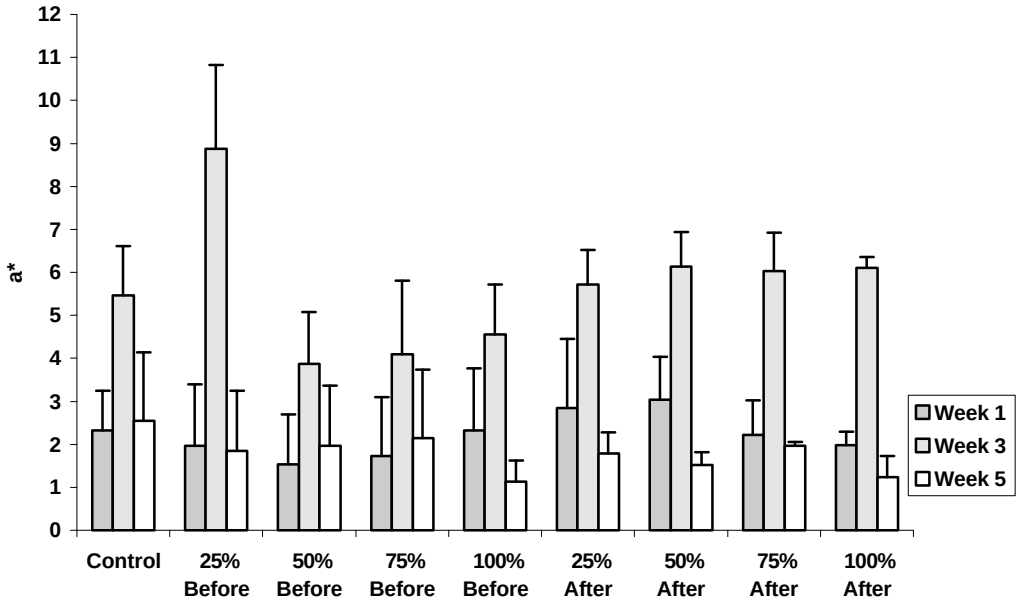


Figure 32. Mean a* values of folic acid fortified fat free strawberry yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

The b* (yellowness) values for lemon and strawberry yogurts are shown in Figures 33 and 34 respectively. Pasteurization was found significant for strawberry yogurts and non-significant for lemon flavored yogurts. Differences in pasteurization in strawberry yogurts is perhaps due to increased syneresis of samples post-pasteurization (Tables 27 and 28). No significant differences were found overall for level of folic acid and overall time of strawberry yogurts. Level of folic acid addition and overall time was significant for lemon yogurts. Strawberries probably masked the yellowness of the samples and reduced variation in overall time.

The C* (chroma) values for lemon and strawberry yogurts are reported in Figures 35 and 36 respectively. Significant differences were found overall for level of folic acid addition and time for lemon yogurts. Overall time was also found significant for strawberry yogurts. A significant interaction was found between pasteurization*level for strawberry yogurts.

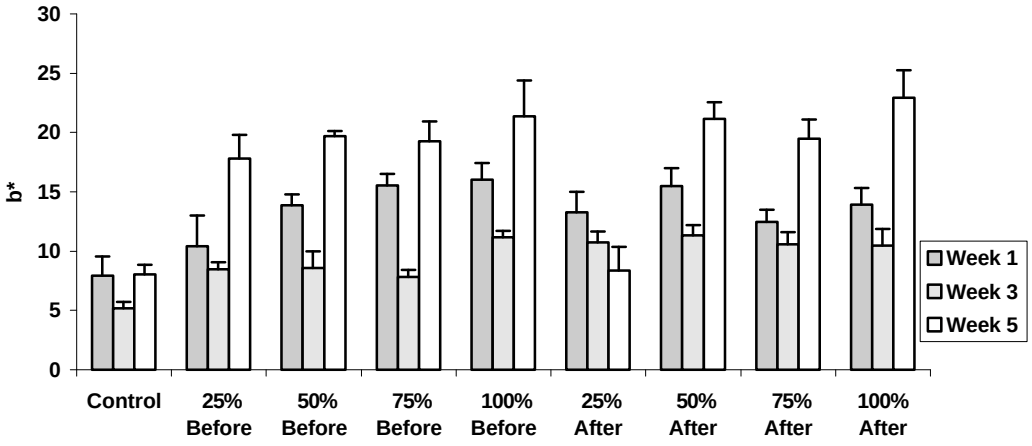


Figure 33. Mean b* values of folic acid fortified fat free lemon yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

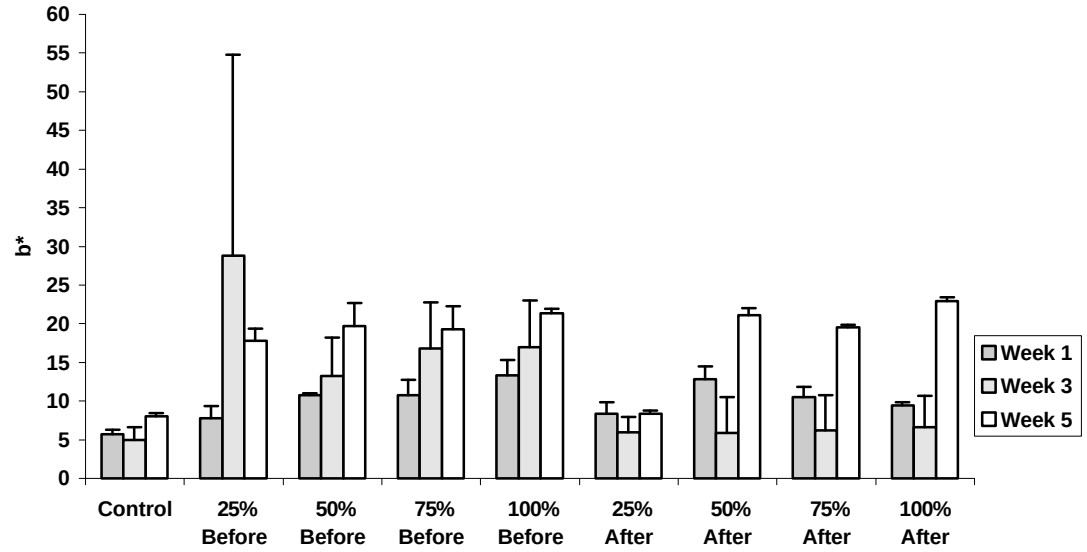


Figure 34. Mean b* values of folic acid fortified fat free strawberry yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

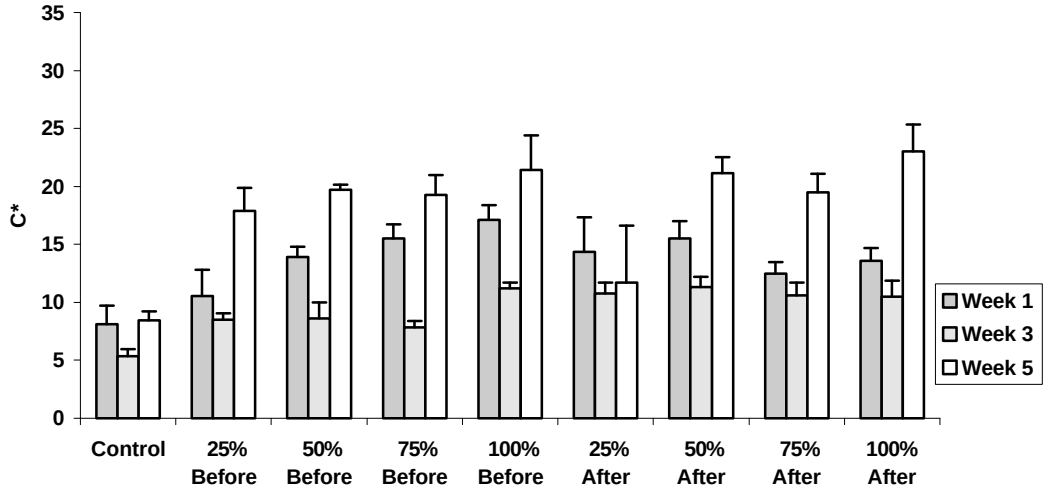


Figure 35. Mean C* values of folic acid fortified fat free lemon yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

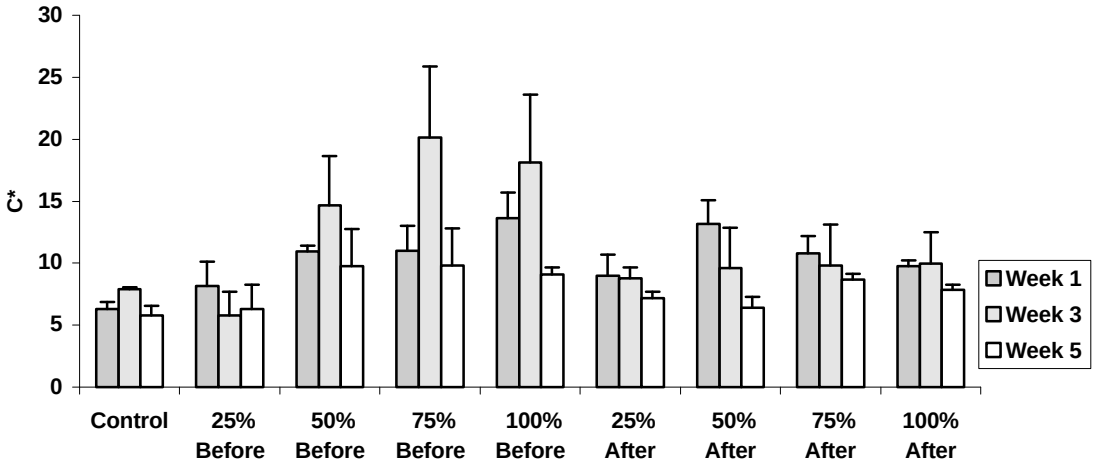


Figure 36. Mean C* values of folic acid fortified fat free strawberry yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

The h (hue angle) values for lemon and strawberry yogurts are reported in Figures 37 and 38 respectively. An h value of 0º is red, 90º is yellow, 180º is green, and 270º is blue. Mean values for lemon yogurts were close to the 90º indicating a yellow color. Mean color values for strawberry were less than lemon indicating a possible masking of the yellow color. Mean values of lemon yogurts were higher (closer to 90º) than plain yogurts indicating a possible effect of the yellow color of the lemon flavor on yogurts. Significant interactions were found for time*pasteurization*level for strawberry yogurts. Level of folic acid addition was found significant for lemon yogurts. Both overall time and pasteurization were found non-significant for lemon yogurts.

Mean flavor scores for lemon and strawberry yogurts are reported in Figures 39 and 40 respectively. Flavor scores are from 1 to 10 with 10 being no criticism. Pasteurization was not significant for lemon or strawberry yogurts. Level of folic acid addition and overall time showed significant differences in mean flavor values

of strawberry and lemon yogurts. As levels of folic acid increased mean flavor scores decreased. Mean scores for lemon and strawberry yogurts were higher than plain.

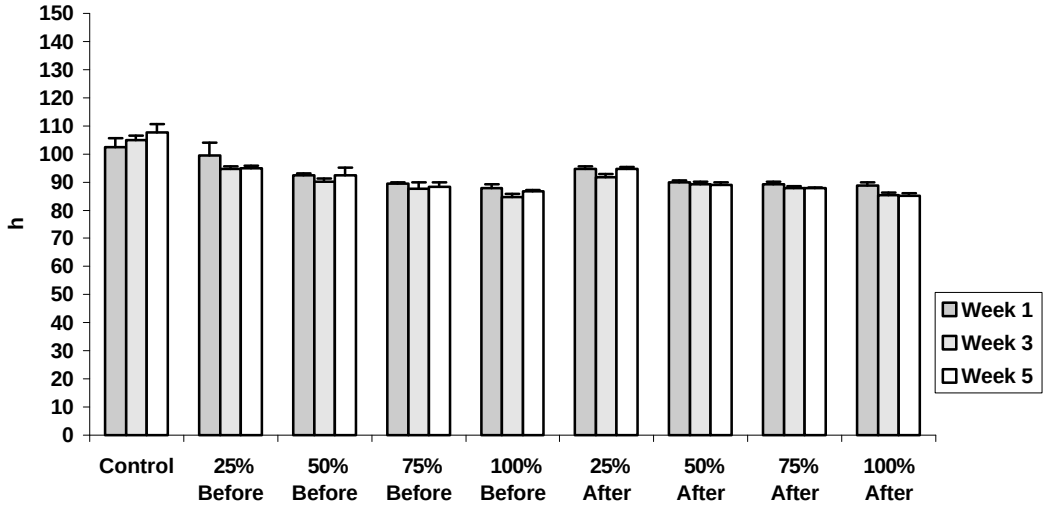


Figure 37. Mean h values of folic acid fortified fat free lemon yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

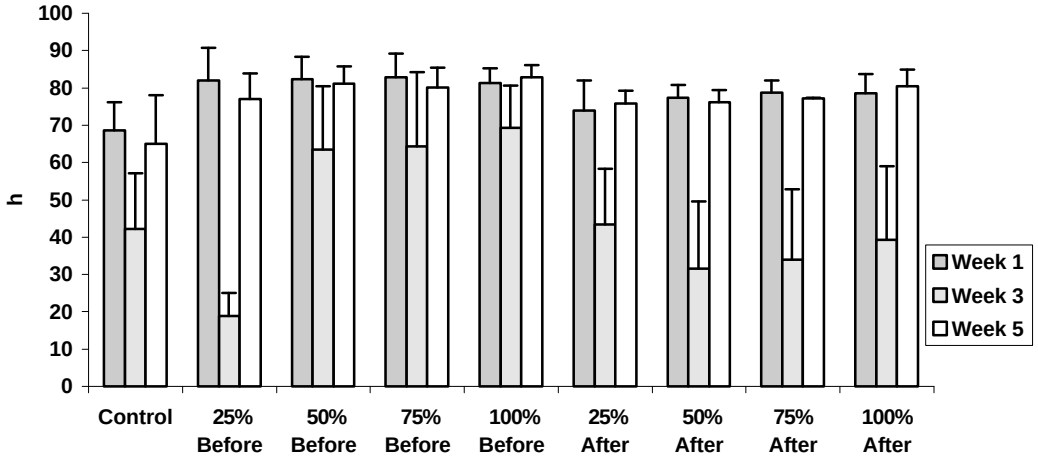


Figure 38. Mean h values of folic acid fortified fat free strawberry yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

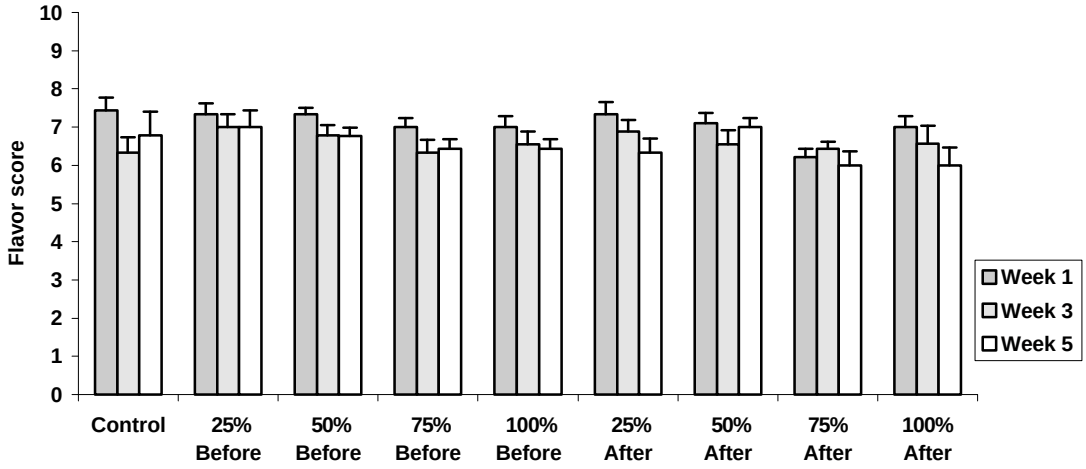


Figure 39. Mean flavor scores of folic acid fortified fat free lemon yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

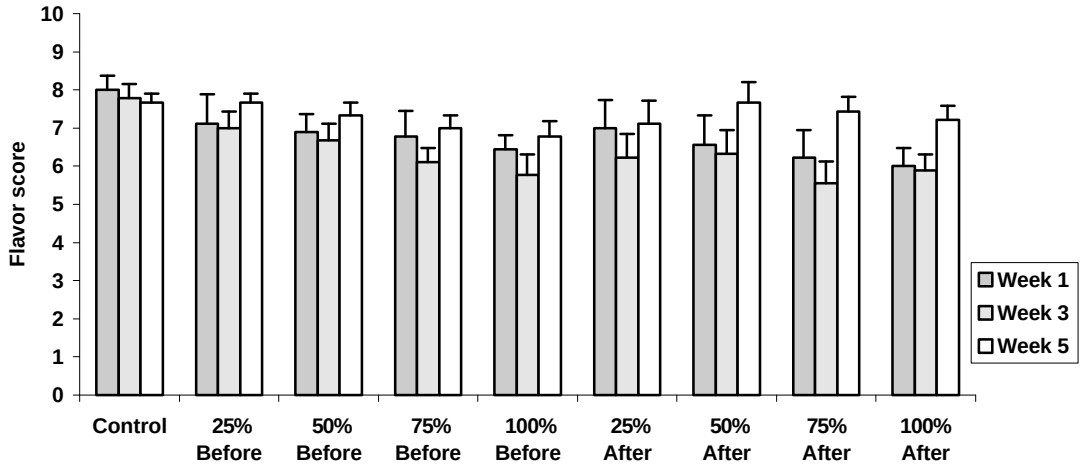


Figure 40. Mean flavor scores of folic acid fortified fat free strawberry yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

Mean body and texture scores for lemon and strawberry yogurts are reported in Figures 41 and 42 respectively. Body and texture scores are from 1 to 5 with 5 being no criticism. A mean value of 5 indicates no defect in body and texture of yogurts. Most values were near 4 indicating little defect in body and texture of yogurts.

Folic acid addition did not appear to impact body and texture scores. Pasteurization and overall time were found significant for lemon and strawberry yogurts. Mean values for both yogurts were lower after pasteurization. Level of folic acid addition was non-significant for both lemon and strawberry yogurts. Results are probably related to syneresis (Figures 25 and 26). Syneresis values were higher for yogurts with folic acid added after pasteurization. Strawberry yogurts had lower mean scores than lemon. This is probably due to the strawberries added to the samples. Mean values appeared to decrease over the storage period.

Mean appearance and color scores for lemon and strawberry yogurts are reported in Figures 43 and 44 respectively. Appearance and color scores are from 1 to 5 with 5 being no criticism. A mean value of 5 would indicate no defect. Level of folic acid addition and overall time was found non-significant for lemon and strawberry yogurts. Pasteurization was found significant for lemon yogurt and non-significant for strawberry yogurt. Mean values for lemon yogurts were lower after pasteurization. This was perhaps because samples appeared to have more released whey.

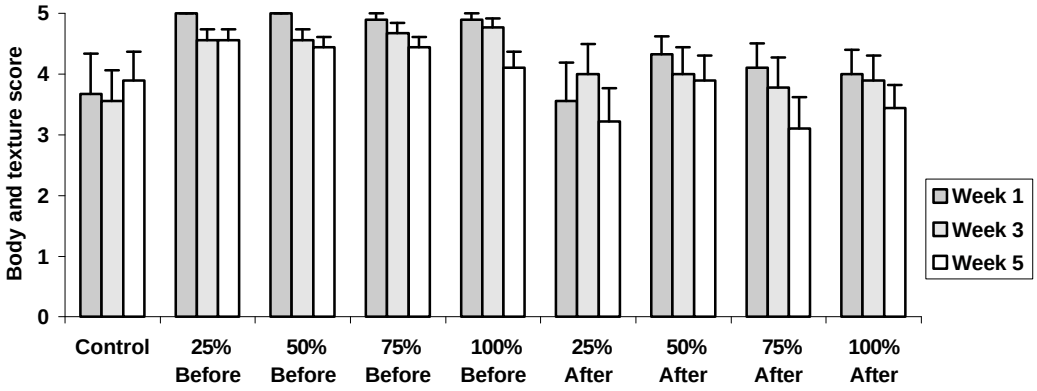


Figure 41. Mean body and texture scores of folic acid fortified fat free lemon yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

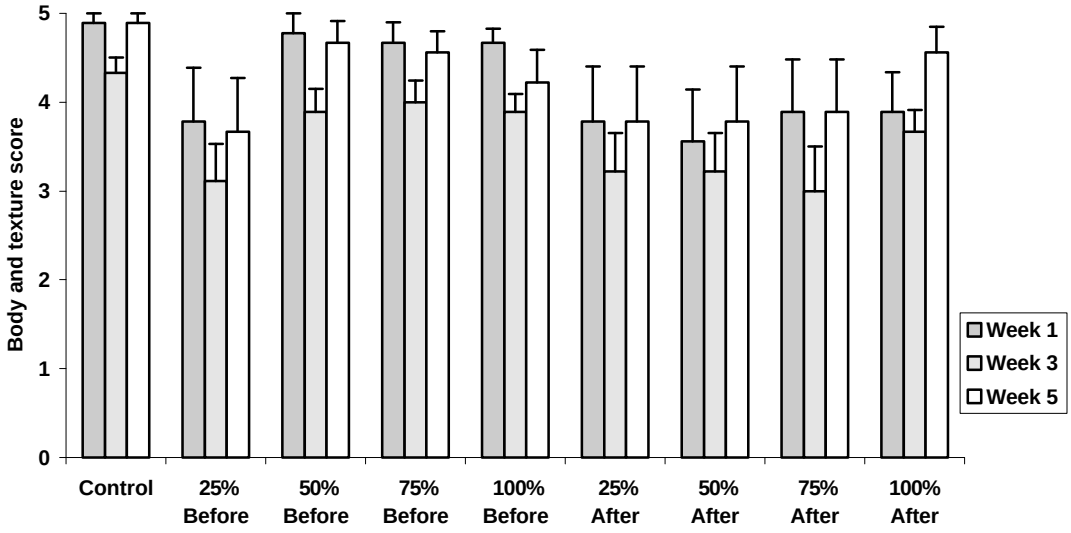


Figure 42. Mean body and texture scores of folic acid fortified fat free strawberry yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

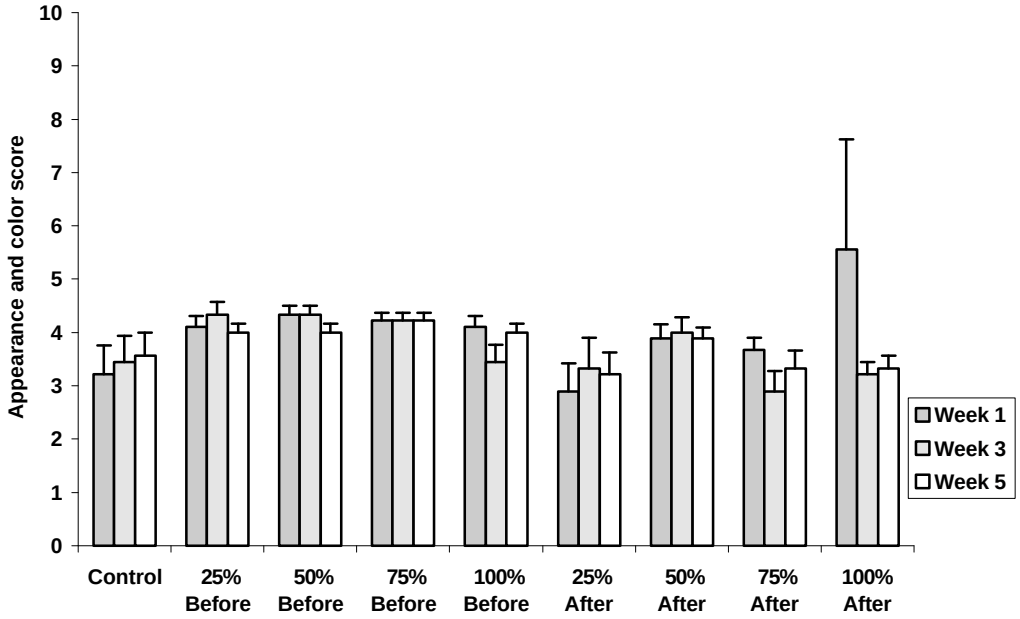


Figure 43. Mean appearance and color scores of folic acid fortified fat free lemon yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

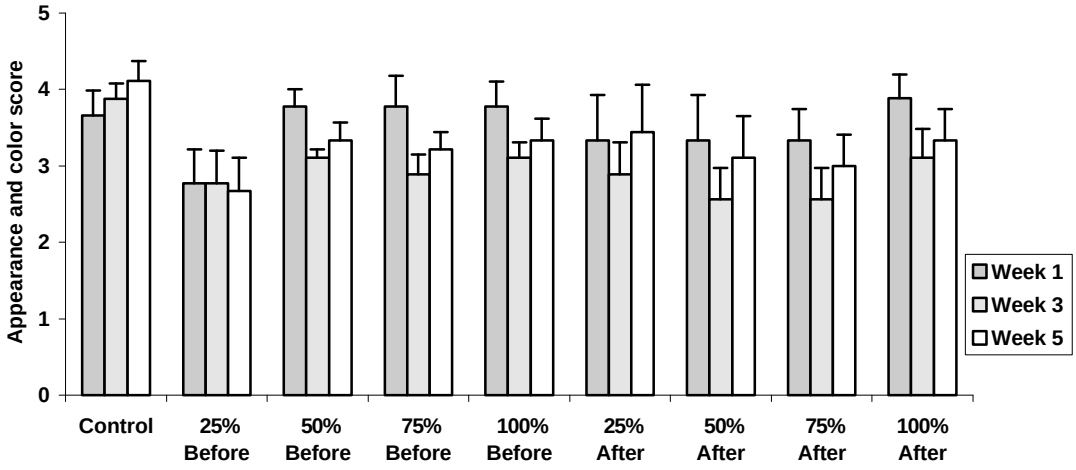


Figure 44. Mean appearance and color scores of folic acid fortified fat free strawberry yogurt at 25%, 50%, 75%, and 100% RDA before and after pasteurization

CHAPTER 8: CONCLUSIONS

Folic acid addition had no effect on protein, fat, moisture, or ash content of lemon or strawberry yogurts. Addition of NaOH helped solubilize folic acid and achieve better peak area results. The pH values of lemon and strawberry yogurts were lower than plain yogurt. Level of folic acid did not impact flavor scores. Mean flavor scores for lemon and strawberry yogurts were higher than plain yogurts. Red color of strawberries to some extent may have helped mask yellowness of samples and reduced b* variation over time. Body and texture values of lemon and strawberry yogurts appeared to decrease over the five week storage period. Addition of flavor to folic acid fortified yogurts helped improve flavor scores which is of use to the dairy industry. Folic acid fortification of flavored yogurts would give industry another product in which to fill consumer demand for products that taste good and have healthful benefits.

Incorporation of folic acid into plain or flavored yogurts will help industry increase sales of dairy products. Consumers are demanding products that have added health benefits without sacrificing taste. Folic acid fortification of yogurt will provide an avenue to accomplish these goals.

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